TITLE OF THE INVENTION

MURINE α(1,3)FUCOSYLTRANSFERASE Fuc-TVII,
DNA ENCODING THE SAME, METHOD FOR PREPARING
THE SAME, ANTIBODIES RECOGNIZING THE SAME,
IMMUNOASSAYS FOR DETECTING THE SAME, PLASMIDS
CONTAINING SUCH DNA, AND CELLS CONTAINING SUCH A PLASMID

BACKGROUND OF THE INVENTION

Field of the Invention:

The present invention relates to murine
α(1,3)fucosyltransferases, Fuc-TVII, DNA encoding such a
murine α(1,3)fucosyltransferase Fuc-TVII, plasmids containing
such DNA, cells transformed with such a plasmid, a method for
producing a murine α(1,3)fucosyltransferase Fuc-TVII by
culturing such cells, monoclonal antibodies which specifically
bind to a murine α(1,3)fucosyltransferase Fuc-TVII, and
immunoassays for detecting a murine α(1,3)fucosyltransferase
Fuc-TVII using such monoclonal antibodies.

Discussion of the Background:

Cell adhesion events between leukocytes and endothelial
cells operate to facilitate the exit of blood leukocytes from
the vascular tree. The selectin family of cell adhesion
molecules, and their counter-receptors, function early in this
process, mediating transient adhesive contacts between
leukocytes and the endothelial cell monolayer. These
selectin-dependent adhesive contacts, together with shear
forces impinging upon the leukocyte, cause the leukocyte to
"roll" along the endothelial monolayer. Leukocyte rolling, in

turn, facilitates subsequent events that include leukocyte activation, firm leukocyte-endothelial cell attachment, and transendothelial migration (T.A. Springer, Cell, vol. 76, pp. 301-314 (1994); R.P. McEver et al, J. Biol. Chem., vol. 270, pp. 11025-11028 (1995)).

E- and P-selectin, expressed by activated vascular endothelial cells, recognize glycoprotein counter-receptors displayed by leukocytes. Each of these selectins can operate to mediate leukocyte rolling in the context of inflammation. L-selectin has also been implicated in mediating leukocyte adhesion to activated vascular endothelium, through interactions with an as yet poorly understood endothelial cell ligand (M.L. Arbones et al, Immunity, vol. 1, pp. 247-260 (1994); K. Ley et al, J. Exp. Med., vol. 181, pp. 669-675 (1995)). By contrast, lymphocyte L-selectin recognizes glycoprotein counter-receptors displayed by specialized cuboidal endothelial cells that line high endothelial venules (HEV) within lymph nodes and Peyer's patches. L-selectin-dependent adhesive interactions in this context operate to facilitate trafficking of lymphocytes (lymphocyte "homing") to such lymphoid aggregates.

The NH_2-terminal C-type mammalian lectin domain common to each of the three selectin family members mediates cell adhesion through calcium dependent interactions with specific oligosaccharide ligands, displayed by leukocytes (E- and P-selectin ligands) (R.P. McEver et al, J. Biol. Chem., vol.

270, pp. 11025-11028 (1995); A. Varki, <u>Proc. Natl. Acad. Sci. U.S.A.</u>, vol. 91, pp. 7390-7397 (1994)), or by HEV (L-selectin) (S.D. Rosen et al, <u>Curr. Opin. Cell Biol.</u>, vol. 6, pp. 663-673 (1994)). Physiological ligand activity for E- and P-selectins is critically dependent on the expression of a non-reducing terminal tetrasaccharide termed sialyl Lewis x (sLe^x) [NeuNAcα2,3Galβ1,4(Fucα1,3)GlcNAc-R] (A. Varki, <u>Proc. Natl. Acad. Sci. U.S.A.</u>, vol. 91, pp. 7390-7397 (1994)), and/or its difucosylated variant (T.P. Patel et al, <u>Biochemistry</u>, vol. 33, pp. 14815-14824 (1994)). However, E- and P-selectins recognize this oligosaccharide in different contexts. P-selectin-dependent cell adhesion is optimal when sLe^x is displayed by serine and threonine-linked oligosaccharides residing on a specific protein termed P-selectin glycoprotein ligand 1 (PSGL-1) (K.L. Moore et al, <u>J. Cell. Biol.</u>, vol. 188, pp. 445-456 (1992); D. Sako et al, <u>Cell</u>, vol. 75, pp. 1179-1186 (1993)). sLe^x-modified PSGL-1 also appears to represent a high affinity counter-receptor for E-selectin (D. Asa et al, <u>J. Biol. Chem.</u>, vol. 270, pp. 11662-11670 (1995); K.D. Patel et al, <u>J. Clin. Invest.</u>, vol. 96, pp. 1887-1896 (1995)). A distinct leukocyte glycoprotein termed E-selectin ligand 1 (ESL-1) (M. Steegmaler et al, <u>Nature</u>, vol. 373, pp. 615-620 (1995)), and its α(1,3)fucosylated, asparagine-linked oligosaccharides, may also function as an E-selectin counter-receptor.

Physiological L-selectin counter-receptors on HEV are represented by the glycoproteins, GlyCAM-1 (L.A. Lasky et al, Cell, vol. 69, pp. 927-938 (1992)), CD34 (S. Baumhueter et al, Science, vol. 262, pp. 436-438 (1993)), and MadCAM-1 (E.L.

5   Berg et al, Nature, vol. 366, pp. 695-698 (1993)). Biochemical studies indicate that L-selectin ligand activity of these molecules is also critically dependent upon post-translational modification by glycosylation. Early studies documented a requirement for sialylation and sulfation

10   (Y. Imai et al, J. Cell Biol., vol. 113, pp. 1213-1221 (1991)), implied a requirement for $\alpha(1,3)$fucosylation, and indicated that these modifications are components of serine and/or threonine-linked glycans. More recent oligosaccharide structural analyses extend this work, and imply that high

15   affinity L-selectin ligand activity depends upon the sulfated variant of the sLe^x determinant, NeuNAcα2,3(SO$_4$6)Galβ1,4(Fucα1,3)GlcNAc-R (S. Hemmerich et al, Biochemistry, vol. 33, pp. 4820-4829 (1994); S. Hemmerich et al, Biochemistry, vol. 33, pp. 4830-4835 (1994); S. Hemmerich

20   et al, J. Biol. Chem., vol. 270, pp. 12035-12047 (1995)).

Expression of sLe^x is determined by cell lineage-specific expression of one or more $\alpha(1,3)$fucosyltransferases (S. Natsuka et al, Curr. Opin. Struc. Biol., vol. 4, pp. 683-691 (1994)). These enzymes utilize the donor substrate GDP-

25   fucose, and catalyze a transglycosylation reaction involving the addition of α1,3-linked fucose to a common 3'-sialyl-N-

acetyl-lactosamine precursor. It can be presumed that expression of the sulfated variant of sLe^x also depends upon lineage-specific expression of $\alpha(1,3)$fucosyltransferase activities operating on sulfate-modified 3'-sialyl-N-acetyl-lactosamine precursors, or that create sLe^x moieties modified subsequently by sulfation.

The identity of the $\alpha(1,3)$fucosyltransferase(s) responsible for selectin ligand expression in leukocytes is not well-defined, and HEV-specific $\alpha(1,3)$fucosyltransferases have not been described. To date, five different human $\alpha(1,3)$fucosyltransferases have been cloned (J.F. Kukowska-Latallo et al, Genes & Dev., vol. 4, pp. 1288-1303 (1990); B.W. Weston et al, J. Biol. Chem., vol. 267, pp. 24575-24584 (1992); B.W. Weston et al, J. Biol. Chem., vol. 267, pp. 4152-4160 (1992); J.B. Lowe et al, J. Biol. Chem., vol. 266, pp. 17467-17477 (1991); S.E. Goelz et al, Cell, vol. 63, pp. 1349-1356 (1990); R. Kumar et al, J. Biol. Chem., vol. 266, pp. 21777-21783 (1991); S. Natsuka et al, J. Biol. Chem., vol. 269, pp. 16789-16794 (1994); K. Sasaki et al, J. Biol. Chem., vol. 269, pp. 14730-14737 (1994)). Northern blot and molecular cloning analyses imply that two of these, termed Fuc-TIV (J.B. Lowe et al, J. Biol. Chem., vol. 266, pp. 17467-17477 (1991); S.E. Goelz et al, Cell, vol. 63, pp. 1349-1356 (1990); R. Kumar et al, J. Biol. Chem., vol. 266, pp. 21777-21783 (1991)) and Fuc-TVII (S. Natsuka et al, J. Biol. Chem., vol. 269, pp. 16789-16794 (1994); K. Sasaki et al, J. Biol.

Chem., vol. 269, pp. 14730-14737 (1994)), are expressed in leukocyte cells, and represent candidates for critical participation in selectin ligand expression. The role of Fuc-TIV (also known as ELAM-1 Ligand Fucosyl Transferase, or ELFT) in this process is not clear, however. While Fuc-TIV/ELFT is able to efficiently utilize non-sialylated N-acetyl-lactosamine precursors to direct expression of the Le^x moiety (J.B. Lowe et al, J. Biol. Chem., vol. 266, pp. 17467-17477 (1991); R. Kumar et al, J. Biol. Chem., vol. 266, pp. 21777-21783 (1991)), this enzyme cannot determine Le^x expression in all cellular contexts (S. Goelz et al, J. Biol. Chem., vol. 269, pp. 1033-1040 (1994)), and its ability to do so in leukocytes, or in leukocyte progenitors, has not been demonstrated. By contrast, Fuc-TVII is apparently able to determine sLe^x expression in all mammalian cellular contexts examined, where sLe^x synthesis is biochemically possible (S. Natsuka et al, J. Biol. Chem., vol. 269, pp. 16789-16794 (1994); K. Sasaki et al, J. Biol. Chem., vol. 269, pp. 14730-14737 (1994)). Neither enzyme has been tested for its ability to participate in the synthesis of L-selectin ligands represented by the sulfated sLe^x determinant.

Thus, there remains a need for additional $\alpha(1,3)$ fucosyltransferases and methods, cells, plasmids, and DNA useful for preparing the same. There also remains a need for antibodies and immunoassays useful for detecting such $\alpha(1,3)$ fucosyltransferases.

SUMMARY OF THE INVENTION

Accordingly, it is one object of the present invention to provide novel $\alpha(1,3)$ fucosyltransferases.

It is another object of the present invention to provide novel DNA encoding such a $\alpha(1,3)$ fucosyltransferase.

It is another object of the present invention to provide novel plasmids containing such DNA.

It is another object of the present invention to provide novel cells transformed with such a plasmid.

It is another object of the present invention to provide a novel method for preparing such an $\alpha(1,3)$ fucosyltransferase by culturing such a transformed cell.

It is another object of the present invention to provide novel monoclonal antibodies which bind specifically to such an $\alpha(1,3)$ fucosyltransferase.

It is another object of the present invention to provide a novel immunoassay for detecting such an $\alpha(1,3)$ fucosyltransferase using such a monoclonal antibody.

It is another object of the present invention to provide a novel method to fucosylate moieties by means of such an $\alpha(1,3)$ fucosyltransferase.

These and other objects, which will become apparent during the following detailed description, have been achieved by the inventors' cloning of $\alpha(1,3)$ fucosyltransferase Fuc-TVII.

BRIEF DESCRIPTION OF THE DRAWINGS

A more complete appreciation of the invention and many of the attendant advantages thereof will be readily obtained as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings, wherein:

Figure 1a shows the structure and functional activities of the murine Fuc-TVII gene and cDNAs. The multi-exon structure of the murine Fuc-TVII gene is shown at top. Numbering below the schematic corresponds to the nucleotide positions of intron-exon boundaries, and the first (1) and last (3520) nucleotides of the known sequence of the locus. Intron-exon boundaries are defined by comparison of the cDNA sequences to the corresponding genomic DNA sequence (see Fig. 2). Numbering above the schematic, immediately above "ATG"s, corresponds to the nucleotide position of the first nucleotide in each of the three potential initiation codons, as discussed in detail below. Numbering above the schematic, immediately above "STOP"s, corresponds to the nucleotide position of the translational termination codon (TGA; base pairs 1900-1902) localized to exon 3b, that truncates the potential open reading frame initiated by the start codon at nucleotide 996-998 in cDNA classes represented by cDNAs 6 and 10 (see text for details). Representative members of the five structurally different classes of Fuc-TVII cDNAs isolated from the murine cytotoxic T-lymphocyte cell line 14-7fd are

schematically represented below the gene structure schematic. The cDNAs shown are the representative member of each class with the longest 5′ extension. The number of cDNAs isolated in each class is indicated in the column labeled "number of cDNAs". Each cDNA was transiently expressed in COS-7 cells (see the Experimental Procedures in the Examples). The transfected COS-7 cells were then subjected to flow cytometry analysis to characterize the cell surface glycosylation phenotype determined by each cDNA. The fraction of sialyl Lewis x-positive cells in the transfected population (positive staining with the monoclonal antibody CSLEX-1) normalized to transfection efficiency as determined by chloramphenicol acetyl transferase activity encoded by a co-transfected plasmid vector encoding this enzyme (see the Experimental Procedures in the Examples) is indicated in the column labeled "% CSLEX-1 positive". These results represent the fraction of antigen-positive cells observed above a background of 2% staining obtained with the negative control vector pCDM8. Extracts were also prepared from the transfected cells, and were subjected to *in vitro* $\alpha(1,3)$ fucosyltransferase assays using 5 mM sialyl-N-acetyllactosamine as an acceptor (see the Experimental Procedures in the Examples). The specific activity of the $\alpha(1,3)$ fucosyltransferase activity encoded by each cDNA (normalized for transfection efficiency) is indicated in the column labeled "Sp. Act. (cpms/μg/hr)".

Figure 1b shows the results of a Western blot analysis of the polypeptides expressed in COS-7 cells by cDNAs 3, 5, 6, 10, and 14. The extracts used in the $\alpha(1,3)$ fucosyltransferase assays shown in Figure 1a, above, were also subjected to Western blot analysis using an antigen-affinity purified anti-Fuc-TVII antibody. The amounts of protein analyzed from each type of transfected cell extract were varied to achieve normalization to the transfection efficiencies, exactly as indicated above in Figure 1a for the flow cytometry and $\alpha(1,3)$ fucosyltransferase activity analyses. Cell extracts were fractionated by SDS-polyacrylamide gel electrophoresis, electro-blotted to a PVDF membrane, and the Fuc-TVII expression vector-encoded polypeptides were identified by probing with an antigen affinity-purified rabbit anti-Fuc-TVII antibody, goat anti-rabbit IgG-peroxidase conjugate, and a commercially available enhanced chemiluminescence reagent (ECL, Amersham), as described in the Experimental Procedures in the Examples;

Figure 2 shows the nucleotide and deduced amino acid sequence of the isolated mouse Fuc-TVII gene. The DNA sequence was derived from a phage containing the murine Fuc-TVII locus. DNA sequence present in cDNAs (Figure 1a) is shown in upper case, whereas DNA sequence corresponding to intronic positions is displayed in lower case. Amino acid sequences predicted by the cDNA sequences are shown in single letter code. As discussed in detail in the text, alternative

splicing events yield different cDNAs that may in turn encode three different polypeptides. One protein is predicted to initiate at the methionine codon localized to nucleotide positions 996-998 (389 residues, 44,492 Da; cDNA 5; Fig. 1).

5　A second protein is predicted to initiate at the methionine codon localized to nucleotide positions 1947-1949 (342 residues, 39,424 Da; cDNAs 6, 10, and 14; Fig. 1). The third protein is predicted to initiate at the methionine codon localized to nucleotide positions 2126-2128 (318 residues,

10　36,836 Da; cDNA 3, as well as all other cDNAs; Fig. 1);

　　　Figures 3a and b show the tissue specific expression patterns of the murine Fuc-TVII gene. Oligo-dT purified mRNA (5 μg) purified from murine tissues and cell lines was fractionated by agarose gel electrophoresis, blotted to a

15　nylon hybridization membrane, and was probed with a 979 base pair DNA segment derived from the coding region of the mouse Fuc-TVII locus (nucleotides 2228-3207; see Fig. 2 and the Experimental Procedures in the Examples). RNA molecular size standards, in kb, are indicated at the left in each panel.

20　Each blot was subsequently stripped and re-probed with a radiolabeled chicken glyceraldehyde 3-phosphate dehydrogenase probe to confirm that RNA samples were intact and loaded in equivalent amounts (see the Experimental Procedures in the Examples);

Figure 3a shows the polyadenylated RNA isolated from mouse tissues, and from the murine T-lymphocyte cell line 14-7fd (14FD).

Figure 3b shows the polyadenylated RNA isolated from mouse bone marrow and spleen, and from cultured murine leukocyte cell lines. Cell lines represent the following lineages: MEL, murine erythroleukemia cell line; P388 and RAW (RAW 264.7), macrophage; EL4, T cell; S107, 63 (TH2.54.63) and 180.1, B cell lines (hybridomas);

Figure 4 shows the results of an *in situ* hybridization analysis of Fuc-TVII transcripts in murine lymph nodes and Peyer's Patches. Sequential 10 micron thick frozen sections of an axillary lymph node (column labeled *Peripheral Lymph Node*; panels A, B, C), a mesenteric lymph node (column labeled *Mesenteric Lymph Node*, panels D, E, F), and Peyer's patches (column labeled *Peyer's Patches*; panels G, H, I), were stained with hematoxylin and eosin (row labeled *H & E*; panels A, D, G, photograph at 5X magnification using bright field illumination), or were processed for *in situ* hybridization, as described in the Experimental Procedures in the Examples. Adjacent sections subjected to *in situ* hybridization were probed with an ^{35}S-labeled anti-sense RNA probe derived from base pairs 2196-2497 of the murine Fuc-TVII locus (row labeled *antisense*; panels B, E, H), or were probed with an ^{35}S-labeled negative control sense RNA probe derived from base pairs 2196-2497 of the murine Fuc-TVII locus (row labeled *sense*; panels

C, F, I). Sections processed for *in situ* hybridization were
photographed at 5X magnification using dark field
illumination. The white areas in panels B, E, and H
correspond to sites (i.e. high endothelial venular endothelial
cells in panels B and E; high endothelial venular endothelial
cells and lumenally-positioned cells [the white-stained "caps"
on the lymphoid aggregates] in panel H) where the Fuc-TVII
antisense probe identifies Fuc-TVII transcripts (see text for
details);

    Figures 5a and b show the immunohistochemical co-
localization of expression of Fuc-TVII, MECA-79, and
L-selectin ligands, in lymphoid aggregate high endothelial
venular endothelial cells.

    Figure 5a shows the co-localized expression of Fuc-TVII,
MECA-79, and L-selectin ligands in HEV. Sequential 10 micron-
thick frozen sections of axillary lymph nodes (column labeled
Peripheral Lymph Node), mesenteric lymph nodes (column labeled
Mesenteric Lymph Node), and Peyer's patches (column labeled
Peyer's Patches) were stained with an antigen affinity
purified rabbit polyclonal anti-murine Fuc-TVII (row labeled
Fuc-TVII), with the monoclonal antibody MECA-79 (row labeled
MECA-79), with a murine L-selectin/human IgM chimera (row
labeled *L-sel/IgM Chimera*), or with the L-selectin/IgM chimera
stained in the presence of EDTA (row labeled *L-sel/IgM Chimera
+ EDTA*). Detection of section-bound primary
immunohistochemical reagents was subsequently accomplished

using secondary immunochemical reagents labeled with flurochromes (FITC-conjugated [green] anti-rabbit IgG for Fuc-TVII; FITC-conjugated [green] anti-human IgM for L-selectin/IgM; rhodamine-conjugated [red] anti-rat IgM for
5 MECA-79), as described in the Experimental Procedures in the Examples. These colored detection schemes yield white area on the black and white reproductions of the original photographs of these experiments. Thus, the "green" and "red" regions appear as white regions. Photomicrographs were taken at 40X
10 magnification, using fluorescent microscopic procedures as described in the Experimental Procedures in the Examples.

Figure 5b is a high power magnification of peripheral lymph node HEV staining with anti-Fuc-TVII. Light gray areas in panel A of Figure 5b correspond to MECA-79 staining. White
15 areas in panel A of Figure 5b correspond to staining with anti-Fuc-TVII antibody. A 10 micron-thick frozen section of an axillary lymph node was stained simultaneously with the antigen affinity purified anti-Fuc-TVII antibody and with anti-MECA-79 antibody (panel labeled "immune antibodies").
20 Section-bound antibodies were detected with fluorochrome-conjugated secondary antibody reagents exactly as described in the description of Figure 5a, and in the Experimental Procedures in the Examples. The immediately adjacent 10 micron-thick frozen section of the same axillary lymph node
25 was instead stained simultaneously with a pair of negative control antibodies (normal rabbit IgG, and normal rat IgM;

panel labeled "non-immune antibodies"), and developed in a
manner identical to that used in the "immune antibodies"
panel. Photomicrographs were taken at 400X magnification,
using fluorescent microscopic procedures described in the
5   Experimental Procedures in the Examples.

        DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS
        Thus, in a first embodiment, the present invention
provides novel enzymes, murine $\alpha(1,3)$ fucosyltransferases,
murine Fuc-TVIIs. Suitably, the enzyme has one of the two
10   amino acid sequences corresponding to the polypeptide encoded
by the gene shown in Figure 1a using one of two different ATG
initiation codons. Thus, in a first embodiment, the present
enzyme has the amino acid sequence corresponding to that
encoded by the DNA corresponding to cDNA 5 in Figure 1 and
15   nucleotide positions 996 to 1149, and 2067 to 3079 in Figure
2. In another embodiment, the enzyme has an amino acid
sequence corresponding to that encoded by the cDNAs 6, 10, or
14 in Figure 1 and having a nucleotide sequence corresponding
nucleotide positions 1947 to 1959, and 2067 to 3079 shown in
20   Figure 2. In these two embodiments, it is to be understood
that the amino acid sequences correspond to:
        (1) that obtained by linking the C terminus of the amino
acid sequence encoded by nucleotide positions 996 to 1149 of
Figure 2 to the N terminus of the amino acid sequence encoded
25   by nucleotide positions 2067 to 3079 of Figure 2; and

(2)   that obtained by linking the C terminus of the amino acid sequence encoded by nucleotide positions 1947 to 1959 of Figure 2 to the N terminus of the amino acid sequence encoded by nucleotide positions 2067 to 3079 of Figure 2.

The present enzyme may also have one of the amino acid sequences described above and in which up to 5 amino acid sequences have been added, deleted, or substituted at the amino terminus of the protein, provided that the enzyme retains its activity. In this context an enzyme is considered to retain its activity if it retains at least 10%, preferably at least ¼, more preferably at least ½, of the specific activity of the native enzyme to transfer fucose from GDP-fucose to 3′-sialyl-N-acetyllactosamine as described in the examples below.

The present invention also provides novel fusion proteins in which any of the enzymes of the present invention are fused to a polypeptide such as protein A, streptavidin, fragments of c-myc, maltose binding protein, IgG, IgM, amino acid tag, etc. Preferably, the polypeptide fused to the present invention is fused to the amino terminus of the present enzyme. In addition, it is preferred that the polypeptide fused to the enzyme of the present invention is chosen to facilitate the release of the fusion protein from a prokaryotic cell or a eukaryotic cell, into the culture medium, and to enable its (affinity) purification and possibly immobilization on a solid phase matrix.

Examples of such fusion proteins include those in which the polypeptide, e.g., protein A, is fused to the glycine residue encoded by nucleotide positions 2067 to 2069 in Figure 2 or to the amino group of the proline residue encoded by nucleotide positions 2162 to 2164 in Figure 2.

In another embodiment, the present invention provides novel DNA sequences which encode murine Fuc-TVII, or a fusion protein according to the present invention. Suitably, the present DNA sequence is any which encodes the two amino acid sequences described above. Thus, in a first embodiment, the DNA sequence is any encoding a polypeptide having the amino acid sequence corresponding to that encoded by the cDNA 5 in Figure 1 and having the sequence corresponding to nucleotide positions 996 to 1149, and 2067 to 3079 in Figure 2. In another embodiment, the DNA sequence is any which encodes a protein having the sequence corresponding to that encoded by the cDNAs 6, 10, or 14 in Figure 1 and having the sequence corresponding to nucleotide positions 1947 to 1959, and 2067 to 3079 shown in Figure 2.

Of course, the DNA sequence may encode a protein which corresponds to any of those described above but in which up to 5 amino acid residues have been added, deleted, or substituted at the amino terminus of the protein, provided that the protein retains its activity. In addition, the DNA sequence may encode any of the present fusion proteins.

In a preferred embodiment, the DNA sequence has the nucleotide sequence corresponding to from position 996 to 1149, and 2067 to 3079 shown in Figure 2. In another preferred embodiment, the DNA sequence has the nucleotide sequence corresponding to from position 1947 to 1959, and 2067 to 3079 in the nucleotide sequence shown in Figure 2.

In these preferred DNA sequences, it is to be understood that the DNA sequence is:

(1) that obtained by linking the 3' terminus of the sequence corresponding to nucleotide positions 996 to 1149 in Figure 2 to the 5' terminus of the sequence corresponding to nucleotide positions 2067 to 3079 in Figure 2; and

(2) that obtained by linking the 3' terminus of the sequence corresponding to nucleotide positions 1947 to 1959 in Figure 2 to the 5' terminus of the sequence corresponding to nucleotide positions 2067 to 3079 in Figure 2.

Another set of preferred DNA sequences includes the DNA having a sequence corresponding to nucleotide positions 996 to 3079 in Figure 2 and the DNA having a sequence corresponding to nucleotide positions 1947 to 3079 in Figure 2.

The cloning of the full length DNA sequence shown in Figure 2 is described in great detail in the Examples below. The shorter length fragments of this sequence as well as the other DNA sequences of the present invention may be obtained by conventional techniques, such as solid state DNA synthesis or site-directed mutagenesis (Maniatis, T., et al, Molecular

Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold
Spring Harbor, NY (1989) and Ausebel, F.M., et al, Current
Protocols in Molecular Biology, John Wiley & Sons, NY (1989)).

In yet another embodiment, the present invention provides
novel plasmids or vectors which contain a DNA sequence
according to the present invention. The present plasmid may
be either a cloning vector or an expression plasmid. Suitable
plasmids or vectors are those obtained by inserting a DNA
sequence of the present invention into a plasmid or vector
such as pCDM8, pcDNA1, pREP8, pCEP4, PTZ18, etc. In the case
of an expression plasmid, the DNA sequence of the present
invention is preferably inserted into the plasmid downstream
from a promoter and in the correct reading frame and
transcriptional orientation. The insertion of a DNA sequence
according to the present invention into any conventional
expression plasmid in the correct reading frame and
transcriptional orientation and the insertion of a DNA
sequence of the present invention into a conventional cloning
vector can easily be accomplished by the skilled artisan using
conventional recombinant DNA technology.

The present invention also provides transformed cells
which contain a plasmid or vector according to the present
invention. Suitable host cells include any mammalian cell.
Preferred host cells include Chinese hamster ovary cells, COS
cells, etc. The transformation of such host cells with a
plasmid or vector according to the present invention may be

carried out using conventional techniques Ausebel, F.M., et al, Current Protocols in Molecular Biology, John Wiley & Sons, NY (1989).

In a further embodiment, the present invention provides a method for producing an enzyme of the present invention by culturing in a culture medium a transformed cell according to the present invention for a time sufficient to produce the enzyme. Preferably, the cell has been transformed with an expression plasmid such as cDNA5, cDNA6, cDNA10, or cDNA 14, shown in Figure 1a, into which one embodiment of the present DNA has been inserted. The particular culture conditions, such as temperature, medium, etc., will depend on the type and identity of the transformed cell. However, the selection of appropriate conditions is well within the abilities of the skilled artisan. For example, suitable culture conditions and media for a variety of cell types are taught in Ausebel, F.M., et al, Current Protocols in Molecular Biology, John Wiley & Sons, NY (1989), which is incorporated herein by reference.

In another embodiment, the present invention provides novel monoclonal or polyclonal antibodies which specifically bind to the present enzymes. Such antibodies may be produced using conventional methods such as described in Harlow, E. et al, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1988), which is incorporated herein by reference. Preferably, the present antibodies are monoclonal antibodies. The present antibodies

may be labelled with any conventional label, such as a radiolabel, a chromophore (e.g., a fluorescent label), or an enzyme (e.g., horseradish peroxidase).

The present invention also provides novel immunoassays for the detection and/or quantitation of the present enzymes in a sample. The present immunoassays utilize one or more of the present monoclonal or polyclonal antibodies which specifically bind to the present enzymes. Preferably the present immunoassays utilize a monoclonal antibody. The present immunoassay may be a competitive assay, a sandwich assay, or a displacement assay, such as those described in Harlow, E. et al, Antibodies. A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1988) and may rely on the signal generated by a radiolabel, a chromophore, or an enzyme, such as horseradish peroxidase.

The DNA sequences, enzymes, plasmids, cells, and methods of the present invention have a number of uses. Thus, the present invention provides previously undiscovered murine DNA sequences that encode specific and heretofore undiscovered protein sequences capable of functioning as a GDP-Fuc:NeuNAcα(2,3)-ß-D-Gal(1,4)-D-GlcNAc α(1,3)-fucosyltransferase. These enzymes, when expressed by the present DNA sequence, function within mammalian cells to generate *de novo* expression of specific cell surface glycoconjugate structures on those cells. These structures are recognized by an antibody against the sialyl Lewis x

determinant {NeuNAcα(2,3)Galβ(1,4)[Fucα(1,3)]GlcNAc-R}, where R is an underlying glycoconjugate-glycoprotein, glycolipid, free oligosaccharide, or hydroxyl group. It has been demonstrated that these enzymes do not generate *de novo* expression of

5 specific cell surface glycoconjugate structures known as the stage specific embryonic antigen I (SSEA-1 or Lewis x; structure Galβ(1,4)[Fuc α(1,3)]GlcNAc), the Lewis a structure, or the sialyl Lewis a structure. This enzyme, when expressed by the cloned DNA sequence described here, has also been shown

10 to function in the enzymatic manner implied in its name, when assayed in extracts prepared from cells that express the DNA sequence. The oligosaccharide products of this enzyme represent fucose linked in alpha 1,3 configuration to the GlcNAc residue of an α(2,3)sialylated "type II" lactosamine

15 acceptor. This product is hereinafter referred to as the sialyl Lewis x determinant. For convenience, this reaction is shown in the equation given below:

NeuNAcα(2,3)Galβ(1,4)GlcNAc ----> NeuNAcα(2,3)Galβ(1,4)[Fucα(1,3)]GlcNAc

20   Fuc-TVII

                  and   sialyl Lewis X
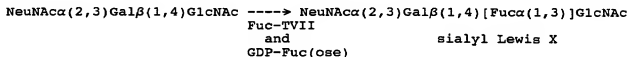
                GDP-Fuc(ose)

The transfer of GDP-fucose to NeuNAcα(2,3)$\underline{Gal}$β[SO$_4$-6]GlcNAc proceeds analogously.

This enzyme may also be able to utilize other

25 oligosaccharide precursors similar or identical to those shown below, wherein the underlined Gal or GalNAc moiety is substituted with another molecule (mono- or oligosaccharide, a

modified sialic acid moiety, or sulfate, for example) and transfer fucose in α(1,3)linkage to the GlcNAc residues on these molecules also referred to hereinafter as sialyl Lewis X for simplicity's sake:

5   Galα(1,3)Galβ(1,4)GlcNAc-R

NeuNAcα(2,3)[GalNAcβ(1,4)]Galβ(1,4)GlcNAc-R

NeuNAcα(2,3)GalNAcβ(1,4)GlcNAc-R

N-glycolylNeuα(2,3)Galβ(1,4)GlcNAc-R

SO₄-3-Galβ(1,4)GlcNAc-R

10   NeuNAcα(2,3)[SO₄-6]Galβ(1,4)GlcNAc-R

NeuNAcα(2,3)[SO₄-6]Galβ(1,4)[SO₄-6]GlcNAc-R

wherein R is a N-linked or O-linked oligosaccharide moiety or backbone.

The location of the catalytic domain of the present

15   enzyme is inferred by comparison to the catalytic domain of several structurally similar enzymes. Specific utilities include:

i. Construction of animal cell lines with specific capabilities with respect to post-translational modification

20   of the oligosaccharides on cell-surface, intracellular, or secreted proteins or lipids by sialyl Lewis x determinants that represent the products of this enzyme (for the production of diagnostics and therapeutics by the biotechnology industry).

Specifically, the cloned DNA sequences described here may
be introduced by standard technologies into a mammalian cell
line that does not normally express the cognate enzyme or its
product (sialyl Lewis x determinants on oligosaccharides), but

5   which <u>does</u> maintain expression of the appropriate
oligosaccharide precursors(s), and GDP-fucose, and transcribed
in that cell in the "sense" direction, to yield a cell line
capable of expressing sialyl Lewis x determinants on
oligosaccharides on cell-surface, intracellular, or secreted

10   proteins or lipids. Alternatively, these cloned DNA sequences
may be introduced by standard technologies into a mammalian
cell line that does express the cognate enzyme and its product
(sialyl Lewis x determinants), and transcribed in that cell in
the "anti-sense" direction, to yield a cell line incapable of

15   expressing sialyl Lewis x determinants on cell-surface,
intracellular, or secreted proteins or lipids. The
introduction and "anti-sense" transcription of the present DNA
sequences in a cell may be carried out using conventional
methods as described in Maniatis, T., et al, Molecular

20   Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory
Press, Cold Spring Harbor, NY (1989), which is incorporated
herein by reference. Alternatively, the endogenous GDP-
Fuc:NeuNAcα(2,3)-ß-D-Gal(1,4)-D-GlcNAc α(1,3)-
fucosyltransferase gene(s), in a mammalian cell expressing the

25   cognate enzyme(s), might be inactivated with the present DNA
sequence by homologous recombination techniques, or by "anti-

sense" oligonucleotide approaches using the present DNA
sequences, or by dominant negative mutant fucosyltransferase
sequences that inactivate endogenous GDP-Fuc:NeuNAcα(2,3)-β-D-
Gal(1,4)-D-GlcNAc α(1,3)-fucosyltransferase(s), derived via

5   mutagenesis and genetic selection schemes, from the present
DNA sequences, or from structurally related but otherwise wild
type α(1,3)fucosyltransferases.

This method may be used to construct animal cell lines
that are suitable host cells for the production of diagnostic

10   or therapeutic materials whose usefulness or efficacy depends
upon the specific post-translational modification determined
by the present cloned DNA sequences and their cognate enzymes.
For example, it is known that the biological effectiveness of
many therapeutic proteins or peptides, recombinant or

15   otherwise, depends critically upon the oligosaccharide
structure(s) that are covalently attached to them. The
structure of these oligosaccharides is primarily a function of
the number and kind of glycosyltransferase enzymes that are
found in the cell used to produce these therapeutic products.

20   Animal cells and some yeasts are competent to perform these
glycosylation reactions; however, not all glycosyltransferase
enzymes are produced by every animal cell or yeast, and
therefore, some oligosaccharide structures (including sialyl
Lewis x determinants generated by the present enzyme) are not

25   produced by them. The converse is also true, namely, that
producing cells may express a glycosyltransferase analogous

to, or identical to, the GDP-Fuc:NeuNAcα(2,3)-β-D-Gal(1,4)-D-GlcNAc α(1,3)-fucosyltransferase encoded by the DNA sequence described here. It is likely that sialyl Lewis x determinants may alter the bioactivity (for better or for worse) of natural

5   or recombinant therapeutic or diagnostic agents (glycoproteins or glycolipids) produced by mammalian or other eukaryotic hosts. Eukaryotic host cells that the biotechnology industry uses to produce these recombinant agents may be altered with the DNA sequence information and related information described

10   in this invention, to add sialyl Lewis x determinants to the oligosaccharides on recombinant products by expressing all or part of the cloned sequences described here in the desired host. Alternatively, sialyl Lewis x determinants may be eliminated from the product produced in these host cells by

15   the use of transfected "anti-sense" vector constructs, recombination-based gene inactivation, "anti-sense" oligonucleotide approaches, or dominant negative mutant fucosyltransferases, outlined above.

The old "methods" used for this process include an

20   empirical approach to identify a cell line that does or does not express this particular enzyme or an enzyme that functions in a similar or identical manner, for the production of the appropriately modified recombinant or natural product. This is not always optimal since cell lines with this particular

25   post-translation modification capabilities may not exist naturally, or may not be especially suited to high level

production of an appropriately modified product.
Alternatively, unwanted sialyl Lewis x determinants present on
a therapeutic material produced by an empirically identified
animal cell line must be removed chemically or enzymatically,
5   a process that may be costly or inefficient. The advantages
of using the cloned, functional present DNA sequences in
conjunction with the technologies outlined above, relative to
these older methods include the ability to construct lines
that specifically lack the capability to generate sialyl Lewis
10   x determinants on the oligosaccharides of glycoproteins or
glycolipids. Properly constructed, these cell lines will
eliminate any need for chemical or enzymatic treatment of a
therapeutic or diagnostic material to remove unwanted sialyl
Lewis x determinants. Moreover, in the event that sialyl
15   Lewis x determinants are found to be desirable for a
particular diagnostic or therapeutic product produced by
animal cells, cell lines may be engineered with the present
cloned DNA sequence to generate these residues.

     ii. Isolation of reagents suitable for efficient
20   enzymatic synthesis and production of oligosaccharides (in
enzyme reactors, for example).

     Oligosaccharides may have therapeutic utility as
immunomodulatory reagents in the field of organ
transplantation. In particular, soluble and solid-phase
25   oligosaccharides may find use as therapeutic agents with which
to block or ameliorate antibody-mediated organ transplant

rejection in cases involving incompatibility due to
differences in the major blood group antigen systems of the
organ donor and the recipient, including the Lewis blood group
system. Likewise, soluble oligosaccharides may find use as
therapeutic agents that function by blocking attachment of
bacterial, viral, or parasitic pathogens to glycoconjugate
"receptors" found on the surface of the animal tissues that
these pathogens invade. For example, there is evidence that
portions of the Lewis blood group oligosaccharide antigens
serve as "receptors" for some forms of uropathogenic bacteria.
Moreover, such glycoconjugates have been implicated in
modulating adhesive events between cells, and between cells
and their environment during developmental and differentiation
processes. These events include binding of spermatozoa to
eggs, and the initial events that mediate attachment of
fertilized ova to the uterine wall at the beginning of
implantation. These observations suggest, for example, the
possibility that contraceptive uses for (biologically
"natural") oligosaccharide molecules might exist. In
addition, specific glycoconjugates containing sialyl Lewis x
determinants have been implicated as ligands for the Selectin
family of adhesion molecules, that play important roles in
mediating adhesion between cells of the immune system, and
some tumor cells, and the surfaces of the endothelial cells
that line the vascular tree. Published observations confirm
that the cloned fucosyltransferase sequence described here

could be used to construct oligosaccharide-type molecules,
with pharmaceutical properties possessing anti-inflammatory
and anti-tumor metastatic functions, and that can prevent
life-threatening tissue damage in acute lung disease (ARDS)
and in tissue re-perfusion, after myocardial infarction, for
example (see, e.g., Buerke, M., et al, J. Clin. Invest., vol.
93, pp. 1140-1148 (1994); Mulligan, M.S., et al, Nature, vol.
364, pp. 149-151 (1993); and Mulligan, M.S., et al, J. Exp.
Med., vol. 178, pp. 623-631 (1993)).

Currently, pharmaceutically useful amounts of
oligosaccharides containing sialyl Lewis x determinants are
produced by chemical synthesis (a procedure that is
inefficient and costly), by isolation from natural sources
(using costly and inefficient procedures that often require
the processing of large quantities of animal or plant
material, and the purification of the desired oligosaccharide
from other contaminating oligosaccharides), or by
glycosyltransferase-assisted synthetic procedures, using other
recombinant α-(1,3)fucosyltransferases. The invention
described here provides a mechanism to synthesize abundant
quantities of purified GDP-Fuc:NeuNAcα(2,3)-β-D-Gal(1,4)-D--
GlcNAc α(1,3)-fucosyltransferase. This could be used to
construct an enzyme bioreactor (enzyme in solution or
immobilized on a solid phase matrix, for example via the
protein-A moiety fused to the catalytic domain of the enzyme,
as described in Ball, et al, J. Am. Chem. Soc., vol. 114, pp.

5449-5451 (1992) capable of enzymatic synthesis of structures containing sialyl Lewis x determinants. This may be more efficient than approaches involving chemical synthesis of structures containing sialyl Lewis x determinants or their

5   purification from natural sources, or by using other known $\alpha(1,3)$fucosyltransferases, for a variety of reasons. One, the only chemicals necessary would be the enzyme substrates; these are easily obtained or synthesized. Two, enzymatic synthesis of such structures will produce only the desired product and

10   the nucleotide diphosphate product of substrate hydrolysis. This latter chemical is found as the natural by-product of these reactions in animal cells, is relatively non-toxic, and may be easily separated from the oligosaccharide synthetic product. By contrast, chemical synthetic procedures typically

15   generate numerous products of side reactions which must be removed, and which may be toxic as well. Similarly, purification of oligosaccharides from natural sources requires the removal of other contaminating oligosaccharides present in the natural material. Three, enzymatic catalysis is

20   extraordinarily efficient; nearly complete conversion of substrate to product can be achieved. By contrast, chemical synthesis of sialyl Lewis x determinants on oligosaccharides is a multi-step process; yields at each step may be much less than 100%, and the cumulative efficiency of current chemical

25   synthesis procedures does not approach the efficiency possible with enzymatic synthesis. Similarly, purification of

oligosaccharides with sialyl Lewis x determinants from natural
materials can entail significant losses inherent to the
purification procedures required to separate the desired
oligosaccharide from contaminating, irrelevant

5   oligosaccharides, with inefficient isolation of the desired
oligosaccharide. The GDPFuc:NeuNAcα(2,3)-ß-D-Gal(1,4)-D-
GlcNAc α(1,3)-fucosyltransferase encoded by the present DNA
sequence has never been previously identified in animal
tissues. In theory, however, this activity may be partially

10   purified from animal tissues for synthetic use. These
purifications are themselves typically inefficient, however,
primarily because such enzymes are typically present in very
low abundance. This invention provides two mechanisms that
may provide for the abundant production of this enzyme.

15   First, this may be done through the construction and selection
of animal cells that produce relatively large quantities of
the enzymes. Alternatively, present nucleic acid sequences
may then be used with standard recombinant DNA technologies to
produce large quantities of glycosyltransferases in mammalian

20   host cells, fungi, yeasts, or using other eukaryotic cell-
based systems (i.e. baculovirus mammalian host), or in
prokaryotic hosts. Furthermore, the sequence encoding this
enzyme may be modified via standard molecular cloning schemes
or mutagenesis to yield a recombinant fucosyltransferase with

25   novel properties that make it more desirable than the wild-
type enzyme. For example, the modifications might be made to

the enzyme that make it more stable, more suitable for immobilization in a bioreactor, or more catalytically efficient for a particular substrate.

   iii.   Isolation of reagents suitable for producing recombinant GDP-Fuc:NeuNAcα(2,3)-ß-D-Gal(1,4)-D-GlcNAc α(1,3)-fucosyltransferase to be used directly as a research reagent, or to be used to generate antibodies against the GDP-Fuc:NeuNAcα(2,3)-ß-D-Gal(1,4)-D-GlcNAc α(1,3)-fucosyltransferase, for research applications.

   The present invention provides two methods for producing large quantities of the present enzymes (see ii. above - i.e. specially constructed animal cells, or via natural or synthetic genes encoding these enzymes) which may be used as a research tool with which to study the structures and functions of oligosaccharides and glycoproteins. Likewise, the enzymes produced by this method, or the nucleic acid sequences and derived protein sequences provided by this method, may be used to generate antibodies to this enzyme (via synthetic peptides or recombinant protein). These antibodies might also be used as research reagents to study the biosynthesis and processing of these enzymes, and might be used as an aid in their purification for all the uses described herein.

   iv. Antibodies to glycosyltransferases as diagnostic reagents.

Aberrant expression of $\alpha(1,3)$ fucosyltransferases has been associated with malignancy in humans, indicating that this enzyme may serve as a tumor marker for early detection of malignancy involving a number of human tissues. Enzyme tumor markers have typically been assayed in body fluids by activity assays, which may be subject to non-specificity due to competing glycosyltransferase activity. These assays may also be insensitive since it is possible that inactive enzymes might be useful as tumor markers but would not be detected by enzyme activity assays. This invention provides a mechanism for generating antibodies to this enzyme (monoclonal and polyclonal antibodies against synthetic peptides constructed from information derived from cloned DNA sequence encoding GDP-Fuc:NeuNAcα(2,3)-ß-D-Gal(1,4)-D-GlcNAc α(1,3)- fucosyltransferase, or against the recombinant enzyme produced by eukaryotic or prokaryotic hosts). Antibodies specific for this GDP-Fuc:NeuNAcα(2,3)-ß-D-Gal(1,4)-D-GlcNAc α(1,3)- fucosyltransferase so produced may be used to detect and quantitate this glycosyltransferases in body fluids, with specificity and sensitivity exceeding enzyme activity assays, serving as a method for the early detection of malignancy.

v. Recombinant enzyme for use in screening natural and synthetic compounds for fucosyltransferase inhibitors or inactivators.

It is known that the sialyl Lewis x determinant is an essential component of counterreceptors for the E-selectin and P-selectin. Related compounds are candidate ligands for L-selectin. These receptor-counter-receptor pairs operate to

5 enable leukocytes (neutrophils, monocytes, eosinophils, lymphocytes in general (in recirculatary events), and some kinds of T lymphocytes) to leave the vascular tree and participate in normal inflammatory events in humans, or in pathological inflammatory events in humans (like ARDS, tissue-

10 reperfusion injury, and a host of other such events). Since the present GDP-Fuc:NeuNAcα(2,3)-ß-D-Gal(1,4)-D-GlcNAc α(1,3)-fucosyltransferase gene is expressed in human leukocytes and plays a central role in sialyl Lewis x biosynthesis, pharmacologic inhibitors of this enzyme will diminish

15 leukocyte sialyl Lewis x expression and thus act as anti-inflammatory pharmaceutical agents for use in humans or other animals in acute and chronic selectin-dependent inflammatory states. The GDP-Fuc:NeuNAcα(2,3)-ß-D-Gal(1,4)-D-GlcNAc α(1,3)-fucosyltransferase described here represents a tool for

20 identifying compounds that inhibit this enzyme, either through "screening" methods to identify such compounds in natural product or chemical libraries (using recombinant enzyme or cell lines expressing this enzyme, in screening assays), or through "rational drug design" strategies (via solution of the

25 enzyme's tertiary structure with the aid of recombinant enzyme, followed by design or identification of molecules that

inhibit the enzyme's catalytic activity or other essential
function).

A number of studies have noted an association between
increased numbers of cell surface sialyl Lewis x determinants
on oligosaccharides of a cell and the ability of that cell to
metastasize in a malignant fashion. If there is a causal
relationship here, then drugs that inhibit the present enzyme
may be used as anti-tumor agents. The reagents described in
this disclosure may prove useful for screening compounds for
antifucosyltransferase activity, since the cloned sequence may
be used with standard techniques to produce relatively large
amounts of pure fucosyltransferase. This will aid in
screening since the effects of potential inhibitors will be
tested on a pure enzyme, without the confounding effects that
may occur in whole cell extracts or with partially purified
enzyme.

vi. Engineering of glycosyltransferase substrate
specificity to generate novel glycoconjugate structures on
secreted or cell-associated glycoconjugates.

The present invention provides a cloned GDP-
Fuc:NeuNAcα(2,3)-ß-D-Gal(1,4)-D-GlcNAc α(1,3)-
fucosyltransferase gene that, when used with appropriate
mutagenesis and genetic selection schemes, may allow the
generation of mutant GDP-Fuc:NeuNAcα(2,3)-ß-D-Gal(1,4)-DGlcNAc
α(1,3)-fucosyltransferases that generate glycosidic linkages

different from that generated by the wild-type enzyme. These novel linkages may or may not be naturally occurring, and could find utility as moieties that enhance bioactivity of the molecules to which they are attached. Directed mutagenesis procedure may also be considered since this enzyme maintains primary sequence similarity to other $\alpha(1,3)$ fucosyltransferases, yet exhibits a distinct set of acceptor substrate utilization properties. Alternatively, mutagenesis and selection approaches may be used to generate mutant GDP-Fuc:NeuNAcα(2,3)-ß-D-Gal(1,4)-D-GlcNAc α(1,3)-fucosyltransferases that act in a dominant negative fashion. The dominant negative mutants so generated may be used to inactivate endogenous glycosyltransferase activities when the product(s) of such an enzyme are not desired. Mutant GDP-Fuc:NeuNAcα(2,3)-ß-D-Gal(1,4)-D-GlcNAc α(1,3)-fucosyltransferases may also be generated, for example, that function as fucosidases that hydrolyze various sugar linkages (fucose, mannose, or others) from oligosaccharides *in vitro* and *in vivo*.

vii. Genotyping individuals at this fucosyltransferase locus.

Absence of a fucosyltransferase similar to the one encoded by the DNA sequence detailed here has been found in several families. Should such absence be associated with a detrimental phenotype, DNA sequence polymorphisms, including

restriction fragment length polymorphisms, within or linked to
the gene corresponding to this cloned gene segment may be used
to genotype individuals at this locus, for the purpose of
genetic counseling. Likewise, the molecular basis for such
detrimental phenotypes might be elucidated via the study of
the gene segment described here, should it be causally-related
to such phenotypes.

viii. Identification and production of inhibitors that
operate as anti-inflammatory pharmaceuticals.

Much effort is being exerted trying to identify compounds
that will inhibit sialyl Lewis x formation in human white
cells, since such molecules, if non-toxic, will act as anti-
inflammatory pharmaceuticals by preventing the synthesis of
sialyl Lewis x in white cells, thus rendering the cells unable
to bind to inflamed vascular endothelium (via selectins)
during inflammation, and thus unable to participate in
extravascular inflammatory activities. One candidate target
for such molecules is the Fuc-TVII enzyme. This is a better
candidate than any of the other known fucosyltransferases
since it is the only known fucosyltransferase that is
expressed in meaningful quantities in white cells and in high
endothelial venules in lymphoid aggregates, that can also
synthesize the sialyl Lewis x determinant. Furthermore, the
leukocytes of mice genetically engineered for a deficiency in
this enzyme are deficient in expression of functional ligands

for E-selectin and P-selectin, and exhibit a concomitant immune deficiency characterized by a deficit in leukocyte mobilization to inflammatory sites. These observations demonstrate that Fuc-TVII controls leukocyte selectin ligand expression, and indicate that inhibition of this enzyme by pharmaceutical agents or maneuvers will represent an anti-inflammatory approach that may have therapeutic benefit in human disease where selectin-dependent inflammation is pathologic. The availability of recombinant Fuc-TVII will facilitate identification of such inhibitory compounds since it may be used in high throughput assays to screen chemical libraries and natural product libraries for inhibitors. Moreover, the recombinant form of the enzyme, or a derivative of the enzyme made via recombinant techniques, may be used to determine its tertiary conformation, including the shape of functionally important surfaces (i.e. GDP-fucose binding pocket, metal binding pocket, oligosaccharide acceptor substrate binding pocket, etc.). One could then use "rational drug design" approaches to identify or synthesize molecules with morphological complementarity that act as inhibitors.

ix. A "one-pot", *in vitro* synthesis of the sialyl Lewis x tetrasaccharide.

The sialyl Lewis x tetrasaccharide, and some derivatives, have been shown to operate as anti-inflammatory molecules in animal models of selectin-dependent inflammation (see, e.g.,

M. Buerke et al, J. Clin. Invest., vol. 93, pp. 1140-1148
(1994); M.S. Mulligan et al, Nature, vol. 364, pp. 149-151
(1993); and M.S. Mulligan et al, J. Exp. Med., vol. 178, pp.
623-631 (1993)). These molecules are now in clinical trials.

5   Chemical synthesis of the sialyl Lewis x molecule is extremely
expensive. Glycosyltransferase-assisted synthetic procedures
have therefore been developed, in which recombinant
glycosyltransferases are used to effect specific and efficient
synthesis of the sialyl Lewis x moiety. These systems involve

10 additional enzymes (not shown in the schemes below) that
continuously form the nucleotide sugar substrates necessary to
the glycosyltransferases (see Ichikawa et al, J. Am. Chem.
Soc., vol. 114, p. 9283 (1992). Because of the nature of the
previously existing enzymes, the conventional synthetic scheme

15 had to proceed in a "two-pot" synthesis (see below), where the
sialyated product of the first pot would have to be separated
from the non-sialylated intermediate product (of Rxn #1), and
then added to a second "pot" where Rxn #3 could take place.
This was necessary in order to avoid a competing reaction

20 (Rxn#4), since the available fucosyltransferases (i.e Fuc-
TIII, Fuc-TV, and Fuc-TVI) would transfer to $Gal\beta(1,4)GlcNAc$
(to form Lewis x) and also to $NeuNAc\alpha(2,3)Gal\beta(1,4)GlcNAc$ (to
form sialyl Lewis x). Since Lewis x cannot be sialylated by
any known $\alpha(2,3)$ sialyltransferases (Rxn #5), the formation of

25 Lewis x would effectively diminish the yield by depleting
substrate for Rxn #2.

```
              Rxn #1                   Rxn #2
            β(1,4)Gal-T            α(2,3)sialyl-T
 Pot #1  GlcNAc -----> Galβ(1,4)GlcNAc ------>NeuNAcα(2,3)Galβ(1,4)GlcNAc


                ┌─────────────────────────────────────────────────────────┐
                │ Rxn #4 (Undesirable: avoided by doing 2 pot synthesis)   │
                │             α(1,3)fucosyl-T                              │
                │ Galβ(1,4)GlcNAc ------>Galβ(1,4)[Fucα(1,3)]GlcNAc        │
                │                                  Lewis x                 │
                │                                                          │
                │                                                          │
                │ Rxn #5 (does not produce a product)                     │
                │                     α(2,3)sialyl-T                       │
                │ Galβ(1,4)[Fucα(1,3)]GlcNAc ----->  No product           │
                └─────────────────────────────────────────────────────────┘


                               Rxn #3
                           α(1,3)fucosyl-T
 Pot #2  NeuNAcα(2,3)Galβ(1,4)GlcNAc ----> NeuNAcα(2,3)Galβ(1,4)[Fucα(1,3)]GlcNAc
                                              sialyl Lewis x
```

This problem can be circumvented with Fuc-TVII. This

enzyme cannot operate on Galβ(1,4)GlcNAc, so it can be

included in the first pot. It will not operate until the

product of Rxn #2 is made, at which time it will complete

5   rxn #3. Thus, Fuc-TVII makes possible the one-pot

synthesis shown below:

One-pot synthesis

```
             Rxn #1                  Rxn #2
           β(1,4)Gal-T            α(2,3)sialyl-T
10   GlcNAc -----> Galβ(1,4)GlcNAc ----->NeuNAcα(2,3)Galβ(1,4)GlcNAc
                                              │  Rxn #3
                                              │  α(1,3)fucosyl-T
                                              │     (Fuc-TVII)
15                                            ▼
                                   NeuNAcα(2,3)Galβ(1,4)[Fucα(1,3)]GlcNAc
                                               sialyl Lewis x
```

        Other features of the invention will become apparent

    in the course of the following descriptions of exemplary

20   embodiments which are given for illustration of the

    invention and are not intended to be limiting thereof.

EXAMPLES

EXPERIMENTAL PROCEDURES

Cell Culture. The sources and growth conditions for
COS-7 cells (K.M. Gersten et al, J. Biol. Chem., vol. 270,
pp. 25047-25056 (1995)), CHO-Tag cells (P.L. Smith et al,
J. Biol. Chem., vol. 269, pp. 15162-15171 (1994)), and
cultured murine blood cell lines (B cell line S107, (M.L.
Atchison et al, Cell, vol. 48, pp. 121-128 (1987)); T cell
line EL4, (L.J. Old et al, Cancer Res., vol. 25, pp. 813-
819 (1965)); B cell hybridoma line TH2.54.63, (T. Hamano et
al, J. Immunol., vol. 130, pp. 2027-2032 (1983)); B cell
hybridoma line 180.1, (M. Hummel et al, J. Immunol., vol.
138, pp. 3539-3548 (1987)); Friend murine erythroleukemia
cell line MEL, (D. Singer et al, Proc. Natl. Acad. Sci.
U.S.A., vol. 71, pp. 2668-2670 (1974); B.L. Weber et al,
Science, vol. 249, pp. 1291-1293 (1990)); macrophage cell
line RAW264.7, (P. Ralph et al, J. Immunol., vol. 119, pp.
950-954 (1977); W.C. Raschke et al, Cell, vol. 15, pp. 261-
267 (1978)); macrophage cell line P388D$_1$, (H.S. Koren et
al, J. Immunol., vol. 114, pp. 894-897 (1975)); and the
cytotoxic T-cell line 14-7fd, (T.J. Braciale et al, J. Exp.
Med., vol. 153, pp. 910-923 (1981); M.E. Andrew et al, J.
Immunol., vol. 132, pp. 839-844 (1984)).

Antibodies. The sources of the monoclonal antibodies
used here have been described previously (anti-Lewis
x/anti-SSEA-1, (D. Solter et al, Proc. Natl. Acad. Sci.

U.S.A., vol. 75, pp. 5565-5569 (1978)); anti-H and anti-
Lewis a, (K.M. Gersten et al, J. Biol. Chem., vol. 270, pp.
25047-25056 (1995)); anti-sialyl Lewis x/CSLEX,
(K. Fukushima et al, Cancer Res.. vol. 44, pp. 5279-5285

5   (1984)); anti-sialyl Lewis a, (D. Chia et al, Cancer Res.,
vol. 45, pp. 435-437 (1985)); anti-VIM-2 antibody, (B.A.
Macher et al, J. Biol. Chem., vol. 263, pp. 10186-10191
(1988)); fluorescein-conjugated goat anti-mouse IgM and IgG
antibodies; Sigma). MECA-79 (P.R. Streeter et al, J. Cell

10   Biol., vol. 107, pp. 1853-1862 (1988)) was the generous
gift of Drs. Louis Picker and Eugene Butcher (Stanford
University).

      cDNA cloning. Mouse Fuc-TVII cDNAs were isolated from
a cDNA library constructed from the mouse cytotoxic T cell

15   line 14-7fd (P.L. Smith et al, J. Biol. Chem., vol. 269,
pp. 15162-15171 (1994)), using colony hybridization
procedures (T. Maniatis et al, Molecular Cloning: A
Laboratory Manual, Cold Spring Harbor Laboratory, Cold
Spring Harbor, NY (1982)) and a segment of the mouse Fuc-

20   TVII gene (Fig. 2) corresponding to nucleotides 2053-2285.

      Murine genomic library screening. Approximately
1.0×10^6 recombinant lambda phage from a genomic library
prepared from mouse 3T3 cell DNA (Stratagene) were screened
by plaque hybridization, using a 324 bp segment of the

25   human Fuc-TIII gene (nucleotides 571-894), and low
stringency hybridization procedures described previously

(K.M. Gersten et al, *J. Biol. Chem.*, vol. 270, pp. 25047-
25056 (1995); K.M. Gersten et al, Doctoral Thesis, pp. 66-
98, University of Michigan, Ann Arbor, Michigan (1995)).
DNA from a phage with a unique restriction pattern was

5   digested with *SacI* and a 2.6 kb fragment which cross-
hybridized with the human Fuc-TIII probe was gel purified
and cloned into the *SacI* site of pTZ19R (Pharmacia LKB
Biotechnology, Inc.). A representative subclone containing
a single insert was designated pMFuc-TVII. The DNA

10   sequence of the 2.6 kb insert was determined by the dideoxy
chain termination method (F. Sanger et al, *Proc. Natl.*
Acad. Sci. U.S.A., vol. 74, pp. 5463-5467 (1977)) using T7
DNA polymerase (Sequenase, United States Biochemical Corp.)
and oligonucleotide primers synthesized according to

15   flanking plasmid sequences. Sequence data was used to
design additional synthetic primers which were then
utilized to sequence the remaining portion of the *SacI*
insert in pMFuc-TVII. Sequence analysis was performed
using the sequence analysis software package of the

20   University of Wisconsin Genetics Computer Group (GCG)
(J. Devereux et al, *Nucleic Acids Res.*, vol. 12, pp. 387-
395 (1984)) and the MacVector version of the IBI Pustell
Sequence Analysis Software package (IBI). Sequence
alignments were assembled with the Gap function of the GCG

25   package.

Transfection and analysis of COS-7 cells and CHO-Tag

cells. COS-7 cells were transfected with various Fuc-TVII

expression vectors using a DEAE-dextran transfection

procedure previously described (J.F. Kukowska-Latallo et

5   al, Genes & Dev., vol. 4, pp. 1288-1303 (1990); T. Maniatis

et al, Molecular Cloning: A Laboratory Manual, Cold Spring

Harbor Laboratory, Cold Spring Harbor, NY (1982)). CHO-Tag

cells were transfected with plasmid DNAs using a liposome-

based reagent (N-[2,3-dioleoyloxy)propyl]-N,N,N-

10   trimethylammonium methylsulfate, DOTAP, Boehringer

Mannheim), as modified previously (P.L. Smith et al, J.

Biol. Chem., vol. 269, pp. 15162-15171 (1994)).

Transiently transfected cells were harvested 72 hours

after transfection, and were stained with monoclonal

15   antibodies diluted in staining media, as previously

described (B.W. Weston et al, J. Biol. Chem., vol. 267, pp.

24575-24584 (1992)). Anti-Lewis a, anti-H, and anti-sialyl

Lewis x antibodies were used at 10 μg/ml. Anti-Lewis x was

used at a dilution of 1:1000. Anti-sialyl Lewis a was used

20   at a dilution of 1:500. Anti-VIM-2 antibody was used at a

dilution of 1:200. Cells were then stained with

fluorescein isothiocyanate-conjugated goat anti-mouse IgM

or anti-mouse IgG and subjected to analysis on a FACScan

(Becton-Dickinson) as previously described (B.W. Weston et

25   al, J. Biol. Chem., vol. 267, pp. 24575-24584 (1992)).

Cells were also co-transfected with the plasmid pCDM8-CAT

(P.L. Smith et al, J. Biol. Chem., vol. 269, pp. 15162-
15171 (1994)), and extracts prepared from these cells were
subjected to chloramphenicol acetyltransferase activity
assays (P.L. Smith et al, J. Biol. Chem., vol. 269, pp.
15162-15171 (1994)), to allow for normalization of flow
cytometry and Western blot data to transfection efficiency.

Fucosyltransferase assays. COS-7 cells transiently
transfected with Fuc-TVII expression vectors were harvested
72 hours after transfection, and extracts were prepared
from these cells, exactly as previously described (J.F.
Kukowska-Latallo et al, Genes & Dev., vol. 4, pp. 1288-1303
(1990); B.W. Weston et al, J. Biol. Chem., vol. 267, pp.
4152-4160 (1992)). These extracts were subjected to
$\alpha(1,3)$ fucosyltransferase assays (B.W. Weston et al, J.
Biol. Chem., vol. 267, pp. 24575-24584 (1992)), assembled
in a total volume of 20 μl. Reaction mixtures contained 3
μM GDP-[^{14}C]fucose, 20 mM acceptor (N-acetyllactosamine,
lactose, lacto-N-biose I, 2'-fucosyllactose (Sigma), or
3' sialyl N-acetyllactosamine (Oxford Glycosystems)), 50 mM
cacodylate buffer, pH 6.2, 5 mM ATP, 10 mM L-fucose, 15 mM
MnCl$_2$ and a quantity of cell extract protein sufficient to
yield approximately linear reaction conditions (consumption
of less than 15% of the GDP-fucose substrate) during the
course of the reaction (1 h). Control reactions were
prepared by omitting the acceptor in the reaction mixture,
and values obtained with these reactions were subtracted

from the corresponding acceptor-replete reaction. This
background radioactivity reproducibly represented less than
1% of the total radioactivity in the assays, and
corresponds to free [^{14}C]fucose present in the

5   GDP-[^{14}C]fucose as obtained from the manufacturer.
Identical enzyme preparations were used in assays for the
determination of enzyme activity with different acceptor
substrates.

    Reactions containing neutral acceptors

10 (N-acetyllactosamine, lactose, lacto-N-biose I,
2'-fucosyllactose, all from Sigma) were terminated by the
addition of 20 µl ethanol and 560 µl water. Samples were
centrifuged at 15,000 x g for 5 minutes and a 50 µl aliquot
was subjected to scintillation counting to determine the

15 total amount of radioactivity in the reaction. An aliquot
of 200 µl was applied to a column containing 400 µl of
Dowex 1X2-400, formate form (J.F. Kukowska-Latallo et al,
Genes & Dev., vol. 4, pp. 1288-1303 (1990); B.W. Weston et
al, J. Biol. Chem., vol. 267, pp. 4152-4160 (1992)). The

20 column was washed with 2 ml of water and the radioactive
reaction product, not retained by the column, was
quantitated by scintillation counting. Reactions with the
acceptor NeuNAcα2→3Galβ1→4GlcNAc (Oxford Glycosystems,
Inc.) were terminated by adding 980 µl of 5.0 mM sodium

25 phosphate buffer, pH 6.8. Samples were then centrifuged at
15,000 x g for 5 minutes, and a 500 µl aliquot applied onto

a Dowex 1x8-200 column (1 ml) prepared in the phosphate form. The reaction product was collected and quantitated as previously described (B.W. Weston et al, _J. Biol. Chem._, vol. 267, pp. 24575-24584 (1992)).

5   Generation of rabbit anti-Fuc-TVII antibody. The PCR was used to amplify a segment of the murine Fuc-TVII gene corresponding to the enzyme's "stem" and catalytic domains (J.B. Lowe, _Seminars in Cell Biology_, vol. 2, pp. 289-307 (1991)), using PCR primers corresponding to base pairs
10   2194-2224 and 3053-3085; Fig.2; 5' primer gcgcggatccCACCATCCTTATCTGGCACTGGCCTTTCACC; 3' primer gcgcggatccAGTTCAAGCCTGGAACCAGCTTTCAAGGTCTTC; BamHI sites underlined). The PCR was completed using twenty rounds of amplification consisting of a 1.5 minute 94°C denaturation
15   step and a 2.0 minute 72°C annealing/extension step. The PCR product was subsequently cloned into the _BamHI_ site of the T7 _Escherichia coli_ expression vector pET-3b (F.W. Studier et al, _Methods Enzymol._, vol. 185, pp. 60-89 (1990)). The insert in one clone (termed pET-3b-Fuc-
20   TVIIstem/cat) containing a single insert in the correct orientation was sequenced to confirm that no errors were introduced during DNA amplification. The recombinant Fuc-TVII fusion protein was produced by inducing mid-log phase _E. coli_ (BL21 Lys S) carrying pET-3b-Fuc-TVIIstem/cat with
25   0.4 mM IPTG for three hours (K.M. Gersten et al, Doctoral Thesis, pp. 66-98, University of Michigan, Ann Arbor,

Michigan (1995); F.W. Studier et al, Methods Enzymol., vol.
185, pp. 60-89 (1990)). The bacteria were subsequently
harvested, and lysed by freezing and then thawing the
bacterial suspension. Bacterial genomic DNA was sheared by

5 sonication, followed by separation of soluble and insoluble
material by centrifugation. The Fuc-TVII protein was found
in the insoluble fraction, as determined by SDS-
polyacrylamide electrophoresis (E. Harlow et al,
Antibodies: A Laboratory Manual, Cold Spring Harbor

10 Laboratory, Cold Spring Harbor, NY (1988)).

Recombinant, E. coli-derived Fuc-TVII was fractionated
by SDS-polyacrylamide gel electrophoresis, and segments of
the gel containing Fuc-TVII were excised and used
subsequently as antigen for rabbit immunizations. Rabbit

15 immunization services were purchased (Pel-Freeze
Biologicals; Rogers, AZ). Each of three rabbits were
initially immunized subcutaneously with a total of
approximately 200 μg of Fuc-TVII in pulverized
polyacrylamide gel slices, mixed with complete Freund's

20 adjuvant. Subsequent immunizations were completed in an
essentially identical manner, at 14 day intervals, except
that antigen was administered in incomplete Freund's
adjuvant. Antisera were harvested ten days following the
last of a total of approximately 6 secondary immunizations.

25 Antigen affinity purification of anti-Fuc-TVII
antibody. The insert in pET-3b-Fuc-TVIIstem/cat was

released by digestion with BamHI and was cloned between the
BamHI sites in the *E. coli* expression vector pATH10 (T.J.
Koerner et al, <u>Methods Enzymol.</u>, vol. 194, pp. 477-490
(1991)), to yield a fusion protein derived from the *E. coli*

5   anthranilate synthase sequence, fused in frame to Fuc-TVII
sequence. This recombinant fusion protein was expressed in
E. coli strain DH5α (induction for 6 hr with 0.0125%
indoleacrylic acid in M9 medium). The bacteria were
harvested, washed, and disrupted by treating with lysozyme

10 (3 mg/ml) in 50 mM Tris HCl pH 7.5, 5 mM EDTA, with 0.65%
NP-40, 0.38M NaCl, followed by sonication for 20 sec at the
maximal microtip setting (Vibracell, Sonics and Materials,
Inc., Danbury, CT) (T.J. Koerner et al, <u>Methods Enzymol.</u>,
vol. 194, pp. 477-490 (1991)). Inclusion bodies were

15 washed twice with 50 mM Tris HCl pH 7.5, 5 mM EDTA, were
solubilized by heating to 100°C in 1% SDS, 12 mM Tris HCl,
5% glycerol, 1% 2-mercaptoethanol, and were subjected to
SDS-polyacrylamide gel electrophoresis. The fractionated
proteins were transferred to PVDF membrane (Bio-Rad

20 Laboratories, Hercules, CA) by electroblotting (1 mA/cm^2).
The membrane was then blocked for 4-6 hr at 4°C with PBS
containing 10% bovine serum albumin, and 0.2% Tween-20. A
strip of membrane containing the recombinant Fuc-TVII
fusion protein was incubated overnight at 4°C with 0.5 ml

25 rabbit anti-mouse Fuc-TVII antiserum, diluted with 2.5 ml
of PBS containing 3% bovine serum albumin and 0.2%

Tween-20. The membrane was then washed at room temperature
in PBS, 0.05% Tween-20, sliced into small pieces, and the
bound antibody was eluted by incubating the membrane
fragments on ice for 10 min in 450 µl Tris-Glycine pH 2.5.

5   The supernatant was collected and immediately neutralized
with 100 µl of 1M Tris-HCl pH 8.0. The elution procedure
was completed a second time, and the two eluates were
pooled and used subsequently for immunohistochemical
procedures.

10   Western blot analysis. Cell extracts were prepared
from transfected COS-7 cells 72 h after transfection.
Extracts contained 50 mM Tris-HCl (pH 6.8), 1% SDS, and 10%
glycerol. Extracts were boiled for 3 min immediately after
preparation, and were stored frozen until use. Protein

15 content was determined using the BCA reagent procedure.
Extracts were prepared for SDS-PAGE by adding
dithiothreitol to a final concentration of 0.1 M, and
bromophenol blue to a final concentration of 0.05%.
Samples were then boiled, and fractionated by

20 electrophoresis through a 10% SDS-polyacrylamide gel.
After electrophoresis, the proteins were electrotransferred
to a PVDF membrane (Biorad). The membrane was rinsed, and
then blocked for 12-14 h at 4°C in phosphate buffered
saline, pH 7.4, containing 10% bovine serum albumin and

25 0.2% Tween-20. The blot was washed at room temperature in
phosphate buffered saline, pH 7.4, 0.2% Tween-20, and was

probed with a 1:200 dilution of antigen affinity purified
rabbit anti-Fuc-TVII antibody. The blot was then washed
and probed with a 1:2500 dilution of a horse radish
peroxidase-conjugated anti-rabbit immunoglobulin (Sigma).

5   The blot was then rinsed, exposed to ECL reagent (Amersham,
UK), and subjected to autoradiography.

Northern blot analysis. Total RNA was prepared from
mouse (FVB/N) tissues and cultured cell lines, using
published procedures (T. Maniatis et al, Molecular Cloning:
10 A Laboratory Manual, Cold Spring Harbor Laboratory, Cold
Spring Harbor, NY (1982)). Oligo-dT-purified poly A+ RNA
samples were electrophoresed through 1.0% agarose gels
containing formaldehyde, and were transferred to a nylon
membrane (Hybond-N, Amersham). Northern blots were
15 prehybridized for 2 hours at 42°C in 1X PE, 5X SSC, 0.5%
sodium dodecyl sulfate, and 150 μg/ml sheared salmon sperm
DNA. Blots were hybridized for 18 hours at 42°C in
prehybridization solution containing $\alpha[^{32}P]$-labeled 974 bp
EagI-EcoRI fragment isolated from the insert in pMFuc-TVII.
20 The EagI site is located at nucleotides 2228-2233 while the
EcoRI site spans base pairs 3202-3207. Blots were stripped
in boiling 0.1% SDS and re-hybridized with a chicken
glyceraldehyde 3-phosphate dehydrogenase probe (A.
Dugaiczyk et al, Biochemistry, vol. 22, pp. 1605-1613
25 (1983)) to confirm that RNA samples were intact and loaded
in equivalent amounts.

Construction of a mouse L-Selectin/IgM chimera
histochemical probe. A mouse L-selectin cDNA (M.H.
Siegelman et al, Science, vol. 243, pp. 1165-1172 (1989))
was kindly provided by Dr. Mark Siegelman at the University
5   of Texas Southwestern Medical Center, Dallas, TX. The
extra-cellular domain was truncated at the junction of its
transmembrane domain with an Hph I digest followed by the
litigation of an adaptor. A human IgM cDNA containing the
CH2, CH3, and CH4 domains (kindly provided by Dr. Ernie
10   Kawasaki, Procept Inc.) was ligated to the adaptor modified
end of the L-selectin sequence in a manner that fuses the
open reading frame encoding L-selectin to the open reading
frame encoding the CH2, CH3, and CH4 domains of human IgM.
This fragment was inserted into the vector SRα-PCDM8
15   immediately downstream of the SRα promoter in the sense
orientation with respect to the SRα promoter. This vector
was introduced into COS-7 cells using the DEAE dextran
transfection method (J.F. Kukowska-Latallo et al, Genes &
Dev., vol. 4, pp. 1288-1303 (1990); T. Maniatis et al,
20   Molecular Cloning: A Laboratory Manual, Cold Spring Harbor
Laboratory, Cold Spring Harbor, NY (1982)). Media was
harvested from the transfected cells 3 days after the
transfection, and was replaced with fresh media (DMEM, 10%
FCS, P/S, Q) that was collected 4 days later. The L-
25   selectin/IgM chimera was purified and concentrated

approximately 40-fold by affinity chromatography on goat anti-human IgM agarose.

Immunohistochemistry procedures. Peripheral (axillary) and mesenteric lymph nodes, and Peyer's patches

5   were isolated from mice immediately after sacrifice. These lymphoid tissues were embedded in OCT medium (Tissue-Tek, MILES, Elkhart, IN), sectioned with a Leica 2800N cryostat, and collected on glass microscope slides.

Sections to be stained with anti-Fuc-TVII were fixed

10   in 2% paraformaidehyde in phosphate buffered saline for 20 min on ice. The sections were rinsed with phosphate buffered saline at room temperature, were quenched with 50 mM NH_4Cl in phosphate buffered saline at room temperature, and then rinsed briefly with water. The

15   tissues were then permeabilized with 100% methanol for 20 min on ice, rehydrated in phosphate buffered saline, and then incubated for 30 min at room temperature with blocking solution A (phosphate buffered saline containing 2% goat serum, 0.05% Triton X-100, 0.05% Tween 20). The blocking

20   solution was aspirated, and the sections were incubated overnight at 7°C with antigen affinity purified anti-Fuc-TVII, used at a final concentration of 5μg/ml, in blocking solution A. After the overnight incubation, the anti-Fuc-TVII/blocking solution was removed, the slides were washed

25   with phosphate buffered saline, and were incubated for 1 hr at room temperature with a FITC-conjugated goat anti-rabbit

IgG reagent (Sigma), diluted 1:200 in blocking solution A.
The slides were then washed at room temperature in
phosphate buffered saline, mounted with citifluor
(Citifluor Products, Chemical Laboratory, The University,
5   Canterbury, Kent CT2 7NH), and examined by
immunofluorescence microscopy (Leitz DM RB microscope).

     Sections to be stained with the monoclonal antibody
MECA-79 (P.R. Streeter et al, _J. Cell Biol._, vol. 107, pp.
1853-1862 (1988)) were fixed on ice for 20 min in 2%
10   paraformaldehyde in phosphate buffered saline, washed at
room temperature with phosphate buffered saline, and were
quenched for 20 min at room temperature with 50 mM NH_4Cl in
phosphate buffered saline. The slides were then rinsed
briefly in water, permeabilized with 100% methanol for
15   20 min on ice, rehydrated in phosphate buffered saline, and
then incubated overnight at room temperature with blocking
solution A (phosphate buffered saline containing 2% goat
serum, 0.05% Triton X-100, 0.05% Tween 20). The blocking
solution was then aspirated, and the sections were
20   incubated for 1 hr at 7°C with MECA-79 at a concentration
of 5 μg/ml in blocking solution A. Sections were then
washed extensively with phosphate buffered saline at room
temperature. The washed sections were then incubated for
1 hr at room temperature with a TRITC-conjugated goat anti-
25   rat IgM reagent (Jackson ImmunoResearch, Pennsylvania),
used at a dilution 1:200 in blocking solution A. The

slides were then washed 3 times at room temperature with
phosphate buffered saline, mounted with citifluor, and
examined.

Sections to be stained with the L-selectin/IgM chimera

5   were fixed in 1% paraformaldehyde, 0.1M cacodylate, pH 7.1,
for 20 min on ice, and were then washed with Tris-buffered
saline, pH 7.4. The L-selectin/IgM chimera was applied to
the sections at a concentration 60 μg/ml, in blocking
solution B (Tris-buffered saline, pH 7.4, containing 2%

10   goat serum), supplemented with either 3 mM $CaCl_2$, or with
5 mM EDTA, and were allowed to incubate overnight at 7°C.
Sections were then washed extensively with ice cold Tris-
buffered saline supplemented with 3 mM $CaCl_2$. Sections
were then incubated for 1 hr at 7°C with a biotinylated

15   goat anti-human IgM reagent (Sigma), diluted 1:200 in
blocking solution B, and supplemented either with 3 mM
$CaCl_2$, or with 5 mM EDTA. The sections were then washed
with ice cold Tris-buffered saline supplemented with 3 mM
$CaCl_2$, and were incubated for 1 hr at 7°C with a FITC-

20   conjugated streptavidin reagent (Vector Labs, Burlingame,
CA) diluted 1:200 in blocking solution B supplemented with
3 mM $CaCl_2$. The slides were washed with ice cold Tris-
buffered saline supplemented with 3 mM $CaCl_2$, mounted with
citifluor, and examined by immunofluorescence microscopy

25   (Leitz DM RB microscope).

In situ hybridization procedures. *In situ* hybridization procedures were completed using a modification of published procedures (D.G. Wilkinson *In Situ Hybridization: A Practical Approach*, IRL Press, Oxford

5 University Press, Oxford, UK). Fresh murine axillary lymph nodes, mesenteric lymph nodes and Peyer's patches were embedded in OCT medium (Tissue-Tek, MILES, Elkhart, IN) and quick-frozen in isopentane on liquid nitrogen. Cryostat sections (10 μM) were collected on Superfrost/Plus

10 microscope slides (Fisher Scientific, Pittsburgh, PA), fixed in freshly prepared 4% paraformaldehyde in PBS for 30 min on ice, washed twice in PBS and digested for 5 min at room temperature with 1 μg/ml proteinase K in 50 mM Tris-HCl pH 7.5, 5 mM EDTA. The slides were then washed in PBS,

15 fixed again in 4% paraformaldehyde, rinsed in water, and treated with 0.25% acetic anhydride in 0.1M triethanolamine pH 8.0, for 10 min at room temperature. Acetylation was followed by room temperature washes in PBS, and then in 0.85% NaCl. The slides were then dehydrated in a graded

20 series of solutions of ethanol in water (30%, 50%, 80%, 95%, 100% ethanol). Air dried sections were overlaid with a hybridization solution containing ^{35}S-labeled RNA, in sense or antisense orientation. RNA probes were derived by in vitro transcription procedures, using recombinant T7 or

25 Sp6 RNA polymerases, initiating on the T7 or Sp6 promoter sequences flanking a DNA segment derived from the coding

region of the mouse Fuc-TVII gene (base pairs 2197-2494;

Fig. 2), as subcloned into the vector pCDNAI (Invitrogen).

The hybridization solution contained 50% deionized

formamide, 0.3 M NaCl, 20 mM Tris HCl pH 8.0, 5 mM EDTA, 10

5   mM phosphate buffer pH 8.0, 10% dextran sulphate,

1X Denhardt's solution (T. Maniatis et al, Molecular

Cloning: A Laboratory Manual, Cold Spring Harbor

Laboratory, Cold Spring Harbor, NY (1982)), 0.5 mg/ml yeast

tRNA, 10 mM dithiothreitol, and 10^7cpm/ml of radiolabeled

10   probe. The hybridization solution was sealed over the

sections with a coverslip and DPX mounting media (BDH Lab

Supplies, Poole, England). Hybridization was carried out

for 16 hours at 55°C in a sealed container humidified with

5X Standard Saline Citrate (SSC). After hybridization, the

15   DPX mounting media seal was removed, and slides were washed

at 55°C for 30 min in 5X SSC, 10 mM dithiothreitol, and

then at 65°C for 30 min in formamide wash buffer (50%

formamide, 2X SSC, 20 mM dithiothreitol). The slides were

then washed 4 times at 37°C in 0.5 M NaCl, 10 mM Tris HCl

20   pH 7.5, 5 mM EDTA. Slides were then digested with RNAseA

(1 μg/ml) for 30 min at 37°C, and were washed in formamide

buffer, then in 2X SSC, and then in 0.1X SSC. Slides were

dehydrated in a graded series of solutions of ethanol in

0.3 M ammonium acetate (30%, 50%, 80%, 95%, 100% ethanol),

25   air dried, and were coated with NTB2 liquid emulsion

(Kodak). Following a two to three week exposure time, the

emulsion was developed using procedures suggested by the manufacturer. Sections were then stained with hematoxylin and eosin, and examined and photographed with bright field and dark field modalities, using a Leitz DM RB microscope.

5   RESULTS

A hybridization screen identifies a novel murine α(1,3)fucosyltransferase locus. In an effort to isolate novel murine α(1,3)fucosyltransferase genes, a murine genomic DNA phage library was screened with a probe

10 corresponding to the catalytic domain of the human Lewis α(1,3/1,4)fucosyltransferase (Fuc-TIII) (J.F. Kukowska-Latallo et al, Genes & Dev., vol. 4, pp. 1288-1303 (1990)), using low stringency hybridization conditions (see the Experimental Procedures). One phage was isolated that

15 contained an insert with a translational reading frame sharing approximately 40% amino acid sequence similarity with the amino acid sequences encoded by four previously cloned members of the human α(1,3)fucosyltransferase family (Fuc-TIII-VI) (J.F. Kukowska-Latallo et al, Genes & Dev.,

20 vol. 4, pp. 1288-1303 (1990); B.W. Weston et al, J. Biol. Chem., vol. 267, pp. 24575-24584 (1992); B.W. Weston et al, J. Biol. Chem., vol. 267, pp. 4152-4160 (1992); J.B. Lowe et al, J. Biol. Chem., vol. 266, pp. 17467-17477 (1991); S.E. Goelz et al, Cell, vol. 63, pp. 1349-1356 (1990);

R. Kumar et al, J. Biol. Chem., vol. 266, pp. 21777-21783
(1991)).

To identify transcripts corresponding to this genomic
sequence, a segment of the phage insert representative of
5   the open reading frame was used to probe Northern blots
prepared from mouse cell lines and tissues. Transcripts
corresponding to this probe were identified in the murine
cytotoxic T-cell line 14-7fd (T.J. Braciale et al, J. Exp.
Med., vol. 153, pp. 910-923 (1981); M.E. Andrew et al, J.
10   Immunol., vol. 132, pp. 839-844 (1984)). A cDNA library
constructed from this cell line (P.L. Smith et al, J. Biol.
Chem., vol. 269, pp. 15162-15171 (1994)) was screened by
hybridization with a segment of the phage insert, yielding
16 hybridization positive colonies. The sequences of all
15   16 cDNA clones were determined, as was the sequence of the
corresponding genomic DNA (Figure 1a). Analysis of this
sequence data indicates that this locus yields multiple,
structurally-distinct transcripts derived from alternative
splicing events, and possibly also from alternative
20   transcription initiation events. Five classes of cDNAs
were identified (Figure 1). Analysis of these cDNA
sequences identifies three methionine codons that may
function to initiate translation of an open reading frame
with amino acid sequence similarity to human Fuc-TIII, Fuc-
25   TIV, Fuc-TV, and Fuc-TVI (Figure 2). The positions of
these methionine codons predicts the synthesis of

α(1,3)fucosyltransferases with different cytosolic domains (encoded by exons 2 and/or 3), but with identical Golgi-localized catalytic domains (encoded by exon 4). One relatively abundant class of cDNAs (represented by cDNA 14)

5   maintains an open reading frame initiating at the methionine codon at nucleotide 1947. This reading frame predicts a 342 residue, 39,424 Da type II transmembrane protein, with a hydrophobic, transmembrane segment derived from amino acids 9-31 (Figure 2). An in-frame methionine

10   codon at nucleotide 2126 predicts a 318 residue, 36,836 Da polypeptide that initiates within the hydrophobic, transmembrane segment of the polypeptide predicted by the longer reading frame initiated at nucleotide 1947. A similar structural arrangement is found in two other cDNA

15   classes, represented by cDNAs 6 and 10. However, these two cDNAs differ from cDNA 14 in that they contain an additional upstream exon with a methionine codon corresponding to nucleotide 996. The translational reading frame initiated by this methionine codon is truncated by a

20   termination codon in exon 2 at a position proximal to the methionine codon at nucleotide 1947, and thus cannot generate a polypeptide that shares similarity to the human α(1,3)fucosyltransferases. However, in cDNA 5, absence of exon 2 allows the translational reading frame generated by

25   the methionine codon at nucleotide 996 to continue in frame with sequence in exon 4. This arrangement predicts the

synthesis of a 389 residue, 44,492 Da type II transmembrane
protein, with the same putative transmembrane segment
defined for the protein predicted by cDNA 14 (Figure 2).
Finally, cDNA 3 is representative of a relatively abundant

5   class of cDNAs that each initiate between the splice
acceptor site of exon 4 and the methionine codon at
nucleotide 2126. This class of cDNAs predicts a 318
residue, 36,836 Da polypeptide that initiates within the
transmembrane segment predicted for the proteins

10   corresponding to the other cDNA classes.

Because the polypeptides predicted by these murine
cDNAs share primary sequence similarity to the four human
α(1,3)fucosyltransferases known at the time (Fuc-TIII, IV,
V, and VI), we anticipated that one or more of them would

15   function as an α(1,3)fucosyltransferase. However, because
the murine peptide sequence shares approximately equivalent
sequence similarity to each of these human enzymes, we
expected that it did not represent the murine homologue of
any of them, and consequently named it Fuc-TVII. This

20   appellation has been justified by subsequent work in which
this murine gene has been used to isolate cDNAs encoding
the human Fuc-TVII (S. Natsuka et al, _J. Biol. Chem._, vol.
269, pp. 16789-16794 (1994)).

None of the three putative initiation codons are

25   embedded in a sequence context consistent with Kozak's
rules for translation initiation (Figure 2) (M. Kozak,

Cell, vol. 44, pp. 283-292 (1986)). To determine which, if
any, of these initiation codons and cognate cDNAs function
to encode the predicted polypeptide(s), and to confirm that
this locus encodes an $\alpha(1,3)$ fucosyltransferase, COS-7 cells

5   were transfected with a cDNA representative of each class,
and the transfectants were subjected to assays to (i)
identify cDNA-determined cell surface-localized fucosylated
oligosaccharide antigens, (ii) identify and quantitate the
polypeptides encoded by cDNAs, and (iii) identify and

10 partially characterize cDNA-determined
$\alpha(1,3)$ fucosyltransferase activity in transfectant cell
extracts using in vitro $\alpha(1,3)$ fucosyltransferase activity
assays.

cDNAs representative of three of the five classes

15 (cDNAs 6, 10, and 14) (Figure 1) each determine relatively
high levels of cell surface-localized sLe[X] expression
(35.1%, 21.8%, and 16.5%, respectively, above a 2%
background) when introduced into COS-7 cells by
transfection. cDNA 5 also directs cell surface sLe[X]

20 expression in COS-7 cells, but at a level (9% positive
cells) that is lower than the sLe[X] expression levels
determined by cDNAs 6, 10, and 14. By contrast, none of
these four cDNAs directs expression of Lewis x, Lewis a, or
sialyl Lewis a determinants. These results indicate that

25 one or both of the two potential methionine initiator
codons in each cDNA can efficiently direct translation to

yield α(1,3)fucosyltransferase activity. These observations further indicate that this α(1,3)fucosyltransferase activity can utilize α(2,3)sialylated lactosamine-based glycan structures to

5   form sLe^X determinants, but indicate that the activity does not efficiently utilize neutral type II oligosaccharide Lewis x precursors, nor neutral or α(2,3)sialylated type I precursors to the Lewis a isomers. Since all four of these cDNAs direct qualitatively identical cell surface antigen

10 profiles in COS-7 cells, it seems likely that individually, or together, each directs the expression of polypeptides that individually, or together, maintain essentially identical acceptor substrate specificities (at least for the four antigens examined).

15   In contrast to the results obtained with cDNAs 5, 6, 10, and 14, cDNA 3 does not direct detectable sLe^X expression. This result suggests that the methionine codon at nucleotide 2126 in this cDNA does not efficiently promote initiation of translation of the cognate mRNA, and

20 thus does not encode functionally significant levels of enzyme activity. Alternatively, this cDNA may encode a polypeptide without α(1,3)fucosyltransferase activity.

    Qualitatively identical results were obtained when these five cDNAs were expressed in another cell line (CHO-

25 Tag cells) (P.L. Smith et al, _J. Biol. Chem._, vol. 269, pp. 15162-15171 (1994)) informative for expression of the Lewis

x, and sLe^x determinants. Unlike COS-7 cells, this cell line is also capable of forming the internally fucosylated VIM-2 determinant (NeuAcα2,3Galβ1,4GlcNAcβ1,3Galβ1,4-(Fucα1,3)GlcNAc-R) (B.W. Weston et al, *J. Biol. Chem.*, vol.

5 267, pp. 24575-24584 (1992)). It was found that none of the cDNAs directs expression of the VIM-2 epitope when expressed in the CHO-Tag cells. Considered together, these results indicate that some, though not all, of the cDNAs can encode an α(1,3)fucosyltransferase activity that can

10 catalyze α(1,3)fucosylation of the N-acetylgalactosamine moiety on a terminal α(2,3)sialylated lactosamine unit, but not to internal N-acetylgalactosamine moieties on α(2,3)sialylated polylactosamine precursors, nor to neutral type II precursors.

15 To confirm that the sLex expression efficiency characteristic of each cDNA correlates with the level of expression of the corresponding protein, cell extracts of the transfected COS-7 cells were subjected to Western blot analysis using an affinity purified rabbit polyclonal

20 antibody generated against a recombinant form of the predicted polypeptide (Figure 1b). Cells transfected with cDNAs 6, 10, and 14 express two major forms of the protein, with molecular weights of 35 kDa and 37 kDa. Smaller amounts of several other proteins are also evident in these

25 cells. The amount of immunoreactive protein generated by these three cDNAs correlates with the level of sLex

expression directed by each. This observation indicates
that the relative sLex expression level directed by each is
a function of the efficiency with which each corresponding
mRNA is translated, and thus the relative intracellular
5   accumulation of the cognate polypeptide.

Cells transfected with cDNA 5 also contain multiple
immunoreactive polypeptides (Figure 1b). The most abundant
pair of these proteins migrate more rapidly than do the
proteins detected in cells transfected with cDNAs 6, 10,
10   and 14, yet are approximately similar in quantity to the
immunoreactive protein directed by cDNAs 6 and 10. As cDNA
5 directs lower levels of cell surface sLex expression than
these two cDNAs, it is therefore possible that the lower Mr
immunoreactive polypeptides found in cDNA 5-transfected
15   cells maintain substantially lower specific enzyme activity
than do the proteins encoded by cDNAs 6, 10, and 14, or are
otherwise less able to direct sLex expression in COS-7
cells. Finally, cells transfected with cDNA 3 do not
contain any detectable immunoreactive proteins. This
20   implies that the putative initiator codon at base pair 2126
in this cDNA does not initiate translation of an
immunoreactive product, and is consistent with the
observation that this cDNA does not yield sLex expression
following transfection into COS-7 cells.

25   Conclusions derived from the flow cytometry and
Western blot analyses summarized above are supported by the

results of *in vitro* $\alpha(1,3)$ fucosyltransferase assays
completed on the same cell extracts. These assays
demonstrate that cells transfected with cDNAs 5, 6, 10, and
14 contain enzyme activity that can transfer ^{14}C-labelled

5   fucose from the nucleotide donor substrate GDP-fucose to
the low molecular weight acceptor 3'-sialyl-N-
acetyllactosamine (SLN; NeuNAcα2,3Galβ1,4GlcNAc) (Figure
1a). For each cDNA, the product of this reaction co-elutes
with a radiolabeled sLe[x] tetrasaccharide standard when

10   fractionated by ion suppression amine adsorption HPLC. The
$\alpha(1,3)$ fucosyltransferase activity directed by each of these
four cDNAs does not utilize the neutral acceptor substrates
N-acetyllactosamine or lacto-N-biose I. And, extracts
prepared from cells transfected with cDNA 3 do not contain

15   detectable $\alpha(1,3)$ fucosyltransferase activity when tested
with 3'-sialyl-N-acetyllactosamine, nor when tested with
the neutral acceptor substrates N-acetyllactosamine or
lacto-N-biose I. These results are entirely consistent
with the flow cytometry data summarized above, and indicate

20   that this locus encodes an $\alpha(1,3)$ fucosyltransferase
activity that apparently requires type II acceptor
substrates that are terminally substituted with an $\alpha(2,3)$-
linked sialic acid residue. Considered together, these
results suggest that differential splicing and/or

25   transcriptional initiation events can control the level of
$\alpha(1,3)$ fucosyltransferase activity, and thus cell surface

sLex expression level, through mechanisms that depend on the efficiency with which each transcript is translated.

Transcription of the mouse Fuc-TVII locus is restricted to cells found in the bone marrow and the lung.

5   Northern blot analysis indicates that transcripts corresponding to the Fuc-TVII locus accumulate to detectable levels in only a few tissues in the adult mouse (Figure 3). Abundant transcript accumulation is only observed in the lung and in the bone marrow, with very

10   small amounts evident in the spleen, salivary gland, and skeletal muscle. Northern blot analysis of cultured murine blood cell-type cell lines indicates that the Fuc-TVII transcript is relatively abundant in the mouse cytotoxic T line 14-fd (used to clone the Fuc-TVII cDNAs), and in the

15   mouse T cell line EL4 (L.J. Old et al, Cancer Res., vol. 25, pp. 813-819 (1965)). Less abundant transcript accumulation is evident in the murine macrophage-derived lines RAW (P. Ralph et al, J. Immunol., vol. 119, pp. 950-954 (1977); W.C. Raschke et al, Cell, vol. 15, pp. 261-267

20   (1978)) and P388 (H.S. Koren et al, J. Immunol., vol. 114, pp. 894-897 (1975)). Fuc-TVII transcripts are not evident in the murine erythroleukemia cell line MEL (D. Singer et al, Proc. Natl. Acad. Sci. U.S.A., vol. 71, pp. 2668-2670 (1974); B.L. Weber et al, Science, vol. 249, pp. 1291-1293

25   (1990)), nor in three murine B-lymphocyte lineage cell lines [S107, (M.L. Atchison et al, Cell, vol. 48, pp. 121-

128 (1987)); TH2.54.63, (T. Hamano et al, _J. Immunol._, vol. 130, pp. 2027-2032 (1983)); 180.1, (M. Hummel et al, _J. Immunol._, vol. 138, pp. 3539-3548 (1987))].

Both the marrow and lung maintain several differently-sized transcripts, including two abundant transcripts of approximately 1.6 and 2.2 kb in size, and a fainter transcript at approximately 3.0 kb. These three transcripts are similar in size to the three most abundant transcripts observed in the murine 14-7fd cytotoxic T cell line. These observations suggest that cells in the bone marrow and lung yield alternatively spliced transcripts similar in structure to those characterized by cDNA cloning studies in the 14-7fd cells. These data also suggest that in the marrow, the Fuc-TVII locus is transcribed in cells assigned to the myclold and T-lymphoid lineages, but not in B-lymphoid lineage cell types, and suggest that expression of this fucosyltransferase correlates with selectin ligand expression on myeloid and T-lymphocyte lineage cell types.

Fuc-TVII is expressed in endothelial cells lining the high endothelial venules in peripheral lymph nodes, mesenteric lymph nodes, and Peyer's patches. The identity of the cell types in the lung responsible for the Northern blot signal in that organ was disclosed by _in situ_ hybridization analyses. These studies identified Fuc-TVII transcripts in paratracheal lymph nodes within the extirpated lung, but not in any other cell type. The

pattern of expression in these nodes suggested that the
Fuc-TVII transcripts were localized to the high endothelial
venules within these nodes. When considered together with
recent observations suggesting that a sulfated derivative

5 of the sialyl Lewis x determinant represents a terminal
oligosaccharide moiety found on HEV-specific L-selectin
ligands (S. Hemmerich et al, _Biochemistry_, vol. 33, pp.
4820-4829 (1994); S. Hemmerich et al, _Biochemistry_,
vol. 33, pp. 4830-4835 (1994); S. Hemmerich et al, _J. Biol._

10 _Chem._, vol. 270, pp. 12035-12047 (1995)), detection of Fuc-
TVII transcripts in these HEV suggests a possible role for
this locus in the synthesis of this fucosylated
oligosaccharide, and this in controlling lymphocyte homing.
To further characterize the HEV-specific expression of Fuc-

15 TVII, this was systematically evaluated using _in situ_
hybridization analysis of HEV in peripheral lymph nodes, in
mesenteric lymph nodes, and in Peyer's patches, where L-
selectin ligand expression has been well-characterized
(S.D. Rosen et al, _Curr. Opin. Cell Biol._, vol. 6, pp. 663-

20 673 (1994)). These analyses (Figure 4) indicate that Fuc-
TVII transcripts accumulate to easily detectable levels in
the HEV of all three types of lymphoid aggregates. An _in_
situ hybridization signal is also obtained with the anti-
sense Fuc-TVII probe in a population of cells that line the

25 gut lumenal surface overlying the Peyer's patches.
Although these are presumed to be epithelial cells, and may

represent so-called M cells (R.L. Owen, Gastroenterology, vol. 72, pp. 440-451 (1977)), their precise identity remains unknown.

5   As noted above, not all Fuc-TVII-derived transcripts yield a protein product. Immunohistochemical analyses were therefore used to confirm that the Fuc-TVII transcripts detected in HEV are accompanied by Fuc-TVII polypeptide expression, and to confirm that such expression co-localizes with L-selectin ligand expression. A rabbit

10   polyclonal antibody raised against the Fuc-TVII peptide yields an intracellular staining pattern in the endothelial cells within HEV in all three lymphoid aggregates (Fig. 5). The perinuclear intracellular staining pattern seen with the anti-Fuc-TVII antibody is consistent with the notion

15   that this enzyme is localized to the Golgi apparatus, where it may participate in the synthesis of fucosylated oligosaccharides with L-selectin ligand activity. In each of the three types of lymphoid aggregate, expression of immunoreactive Fuc-TVII co-localizes with expression of

20   epitopes recognized by the MECA-79 antibody, shown previously to stain HEV, and to interfere with L-selectin binding to HEV (P.R. Streeter et al, J. Cell Biol., vol. 107, pp. 1853-1862 (1988)). Fuc-TVII expression also co-localizes with expression of L-selectin ligands on HEV, as

25   detected with a recombinant mouse L-selectin/human IgM chimeric protein. These observations imply that Fuc-TVII

may participate in the synthesis of the sialylated, sulfated, and $\alpha(1,3)$ fucosylated candidate oligosaccharide components of HEV-derived L-selectin ligands.

In an effort to understand the functions of cell
5 surface fucosylated oligosaccharides in animals, we have established a program to isolate murine $\alpha(1,3/4)$ fucosyltransferase genes, to be used initially as reagents to characterize tissue-specific expression patterns of the loci that control expression of cell
10 surface fucosylated oligosaccharides. These reagents, and the information gathered from their application will be used eventually with transgenic approaches to uncover functions of their cognate cell surface fucosylated oligosaccharides, by perturbing their expression patterns.
15 A cross-hybridization approach outlined here yielded a novel genomic sequence that cross-hybridizes with segments derived from the conserved portions of the human Fuc-Ts III, V, and VI genes, in a position corresponding to their catalytic domains. Following the isolation of this
20 murine genomic locus, functional analyses indicated that it encoded an $\alpha(1,3)$ fucosyltransferase, termed Fuc-TVII, with structural features and catalytic activities that were, at the time of its isolation, unique to the $\alpha(1,3)$ fucosyltransferase family. In particular, this locus
25 was the only $\alpha(1,3)$ fucosyltransferase known to maintain a coding region distributed over more than one exon, and the

first fucosyltransferase with multiple distinct initiation
codons with the potential to yield structurally distinct
polypeptides, characterized by different cytoplasmic
domains, but with essentially identical catalytic

5   activities. The catalytic activity of each Fuc-TVII
isoenzyme is characterized by an ability to utilize
α2,3sialylated type II N-acetyllactosamine precursors,
without the ability to utilize neutral type II, neutral
type I, or sialylated type I N-acetyllactosamine

10   substrates. Similar observations have been made for the
human homologue of Fuc-TVII isolated subsequently (S.
Natsuka et al, J. Biol. Chem., vol. 269, pp. 16789-16794
(1994); K. Sasaki et al, J. Biol. Chem., vol. 269, pp.
14730-14737 (1994)). This catalytic specificity, and the

15   leukocyte-specific expression pattern of this gene,
strongly suggest that it plays a pivotal role in the
biosynthetic scheme that yields the α2,3sialylated,
α1,3fucosylated lactosaminoglycans essential to E- and P--
selectin ligand activity. Furthermore, the leukocytes of

20   mice genetically engineered for a deficiency in this enzyme
are deficient in expression of functional ligands for E-
selectin and P-selectin, and exhibit a concomitant immune
deficiency characterized by a deficit in leukocyte
mobilization to inflammatory sites. These observations

25   demonstrate that Fuc-TVII controls leukocyte selectin
ligand expression, and indicate that inhibition of this

enzyme by pharmaceutical agents or maneuvers will represent an anti-inflammatory approach that may have therapeutic benefit in human disease where selectin-dependent inflammation is pathologic.

5 Other observations made in the work described here suggest a role for Fuc-TVII in directing synthesis of the oligosaccharide components of the ligands for L-selectin. As Rosen and colleagues have shown, L-selectin ligands on HEV correspond to O-linked carbohydrate determinants

10 displayed by the mucin-type glycoproteins GlyCAM-1, CD34, and MAdCAM-1 (L.A. Lasky et al, _Cell_, vol. 69, pp. 927-938 (1992); S. Baumhueter et al, _Science_, vol. 262, pp. 436-438 (1993); E.L. Berg et al, _Nature_, vol. 366, pp. 695-698 (1993)). Their earlier biochemical analyses indicate that

15 the oligosaccharides relevant to L-selectin ligand activity are sialylated, sulfated, and possibly fucosylated (Y. Imai et al, _J. Cell Biol._, vol. 113, pp. 1213-1221 (1991)). More recent structural analyses from the Rosen group are consistent with the hypothesis that the capping groups on

20 such olioosaccharides correspond to sulfated versions of the sialyl sLe^X moiety, with sulfate attached via the 6-hydroxyl of the terminal galactose moiety [NeuNAcα2,3($SO_4$6)Galβ1,4(Fucα1,3)GlcNAc-R], or via the 6-hydroxyl of the subterminal N-acetyl-glucosamine moiety

25 [NeuNAcα2,3Galβ1,4($SO_4$6)(Fucα1,3)GlcNAc-R], or both (S. Hemmerich et al, _Biochemistry_, vol. 33, pp. 4820-4829

(1994); S. Hemmerich et al, Biochemistry, vol. 33,
pp. 4830-4835 (1994); S. Hemmerich et al, J. Biol. Chem.,
vol. 270, pp. 12035-12047 (1995)). Non-fucosylated forms
of these structures were also identified, however, and the

5   evidence that fucose is required for activity of
physiological L-selectin ligands remains circumstantial.

The identification of such non-fucosylated structures
suggests the possibility that these sialylated and sulfated
molecules represent acceptor substrates for

10   $\alpha(1,3)$fucosyltransferases expressed in HEV endothelial
cells. Our observation that expression of the Fuc-TVII
locus co-localizes with L-selectin ligand expression in
such cells suggests that Fuc-TVII may operate in this
context. Furthermore, mice genetically engineered for a

15   deficiency in Fuc-TVII are deficient in L-selectin-
dependent lymphocyte homing to lymph nodes, and are
deficient in expression of L-selectin ligand activity on
the high endothelial venules in peripheral and mesenteric
lymph nodes, and in Peyer's patches. These observations

20   demonstrate that Fuc-TVII controls HEV selectin ligand
expression, and indicate that inhibition of this enzyme by
pharmaceutical agents or maneuvers will represent an anti-
inflammatory approach that may have therapeutic benefit in
human disease where L-selectin-dependent lymphocyte

25   trafficking is pathologic. The notion that sulfated and
sialylated lactosamine moieties represent acceptor

substrates for enzymes like Fuc-TVII is supported by
studies suggesting that Fuc-TIII (E.V. Chandrasekaran et
al, _Biochem. Biophys. Res. Commun._, vol. 201, pp. 78-89
(1994)) and Fuc-TV (P.R. Scudder et al, _Glycobiology_, vol.
5 4, pp. 929-933 (1994)) can utilize sialylated, sulfated
lactosamine-type acceptors. Indirect evidence derived from
studies on the biosynthesis of GlyCAM-1 are also consistent
with this hypothesis (S. Hemmerich et al, _Biochemistry_,
vol. 33, pp. 4820-4829 (1994); D. Crommie et al, _J. Biol._
10 _Chem._, vol. 270, 22614-22624 (1995)). However, our results
indicate that the non-sulfated entity
NeuNAcα2,3Galβ1,4GlcNAc is used, _in vitro_, and _in vivo_, by
Fuc-TVII. Biochemical analyses indicate that 6-O sulfation
of the GlcNAc moiety on this sialylated molecule yields a
15 substance that is used effectively by mouse Fuc-TVII. It
therefore remains to be determined if this sulfated
molecule or others are utilized by Fuc-TVII, in HEV
endothelial cells. Thus, while the biosynthetic scheme for
such molecules remains to be defined, it is clear that Fuc-
20 TVII plays an essential role in this pathway.

     There is evidence to suggest that L-selectin expressed
by granulocytes and other leukocytes mediates adhesion of
these cells to activated vascular endothelium through as
yet undefined extracellular endothelial cell counter-
25 receptors (M.L. Arbones et al, _Immunity_, vol. 1, pp. 247-
260 (1994); K. Ley et al, _J. Exp. Med._, vol. 181, pp. 669-

675 (1995)). Since the chemical nature of this counter-receptor(s) is not known, a role for Fuc-TVII in the synthesis of such ligands remains speculative, and is a subject of current exploration in this laboratory. In any event, the demonstration here that Fuc-TVII is co-expressed with L-selectin ligands on HEV, when considered together with previous observations demonstrating that Fuc-TVII is expressed in leukocytes and can direct synthesis of ligands for E- and P-selectin, along with evidence that the Fuc-TVII null mice are deficient in ligands for E-, P- and L-selectin, indicates that Fuc-TVII is a master control locus for the synthesis of ligands for all three selectins, and thus for controlling selectin-dependent leukocyte trafficking.

Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that, within the scope of the appended claims, the invention may be practiced otherwise than as specifically described herein.